THE DEVELOPMENT OF A NOVEL PROTECTING GROUP FOR NATURAL PRODUCT SYNTHESIS

by

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Ph.D.

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1987

To my parents

This thesis is submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Edinburgh. Unless otherwise stated the work described is original and has not been previously submitted, in whole or in part, for any degree at this or any other University.

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ACKNOWLEDGEMENTS

I would like to thank Professor R. Ramage for his advice and encouragement throughout this work. I would also like to acknowledge Dr. R. Attrill, Dr. C. Barren and Mr. D. Thomas for proof reading this text. Thanks also to Dr. R. Valentine and Dr. J. Regan of Wendstone Chemicals plc for technical and financial support.

COURSES ATTENDED

I have attended the following courses:

Organic Departmental Seminars, various lecturers.

Application of X-Ray Crystallography, various lecturers.

Current Topics in Research Chemistry, various lecturers.

Mass Spectroscopy, Professor K.R. Jennings.

Topics in Medicinal Chemistry, various lecturers.

Cambridge Structural Database, various lecturers.

Perspectives in Cell Biology, Dr. J. Philips.

Research in the Pharmaceutical Industry, ICI/Beecham Pharmaceuticals.

Computing, various lecturers.

ABSTRACT

The use of 2,2-bis(4-nitrophenyl)ethanol (BnpeOH) has led to the development of a novel urethane-type protecting group, 2,2-bis(4-nitrophenyl)ethoxycarbonyl (Bnpeoc). Studies have shown that this group is stable towards acids and tertiary amines whilst rapid and efficient cleavage can be effected by 1,5-diazabicyclo-[4.3.0]non-5-ene (DBN) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) with or without an equivalent of acetic acid. Nineteen amino acids have been protected using either the chloroformate (Bnpeoc-Cl) or the N-succinimidyl carbonate (Bnpeoc ONSu) of this group. These novel protected amino acids have been used to synthesise small peptides both in solution and solid phase. A highly efficient method for anchoring these protected amino acids to a p-alkoxybenzylalcohol resin that avoids racemisation or dimer formation has also been developed.

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Commonly used abbreviations referred to throughout the text

Bnpe	-	bis(4-nitrophenyl)ethyl
Bnpeoc	-	bis(4-nitrophenyl)ethoxycarbonyl
Bnpeoc ONSu	-	bis(4-nitrophenyl)ethyl-N-succinimidyl carbonate
DBN	-	1,5-diazabicyclo[4.3.0]non-5-ene
DBU	-	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	-	dichloromethane
Dioxan	-	1,4-dioxan
DMAP	-	4-N,N-dimethylaminopyridine
DMF	-	dimethylformamide
NMM	-	N-methylmorpholine

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CHAPTER 1

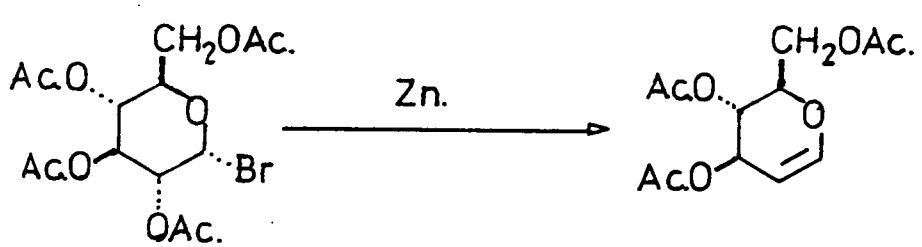
1.1 INTRODUCTION

The role of β-elimination in protection group strategy over the past decade has grown considerably. The advantages offered by β-eliminating groups, due to their removal under non-nucleophilic and essentially neutral or mildly basic conditions, have increasingly been recognised for protection of sensitive functionalities (such as peptides (Sect.1.4), glycopeptides[1,2], β-lactams and nucleotide synthesis (Sect.1.5)). Their unique removal conditions also facilitate their use in association with more conventional acid-labile groups, thereby ensuring relatively mild overall deprotection conditions (Sect.1.4.4).

1.2 Historical background

β-Elimination in organic chemistry has its origins with the olefin synthesis of E. Fischer[3] in 1914.

Fig.1.1

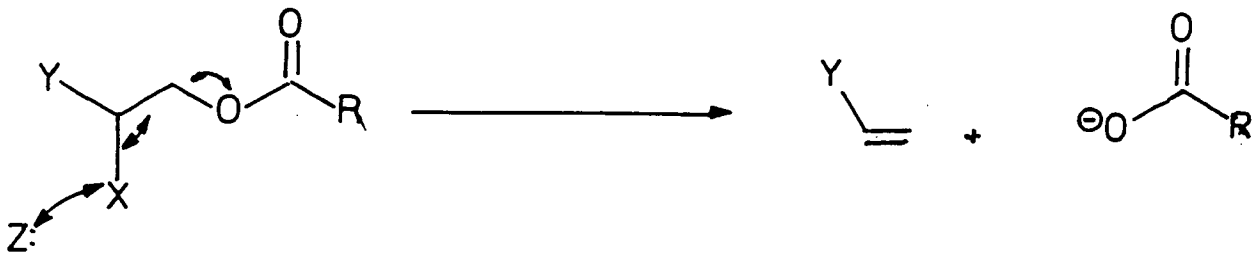


(1) (2)

Later work by Ingold and Hanhart[4] in 1927 using β-arylethyl compounds laid the foundations for the physical organic chemistry study of the elimination process, which

has been researched subsequently (Sect.1.3).

Fig.1.2



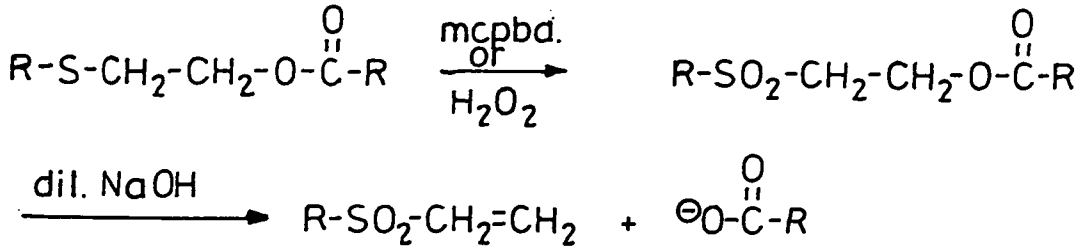
R = aryl, alkyl-: carboxylic acid

= NH -: carbamic acid, decomposes to amine

1.2.1 β-Ar(alk)ylsulphonyl(thio)ethyl groups

C.J.M. Stirling used these results to suggest[5] the use of p-toluenesulphonylethoxycarbonyl as a group for amine[6] and carboxyl[7] protection in peptide synthesis. Removal was facilitated with dilute sodium hydroxide, sodium carbonate or with organic bases[8]. The functionally similar 2-thiomethyl- and 2-sulphonylmethylethyl groups introduced by Rydon and Hardy[9,10] for carboxyl and amine[10b] protection were removed with sodium hydroxide. In the case of 2-thiomethylethyl esters prior oxidation[11] to the sulphone or conversion to the sulphonium salt was required (see 1.2.2) to allow the β-elimination to proceed. The p-nitrophenyl-, and 2,4-dinitrophenylthioethyl derivatives were introduced by Amarql[12,13] and also rely on oxidation to the sulphone for removal, see Fig.1.3.

Fig.1.3

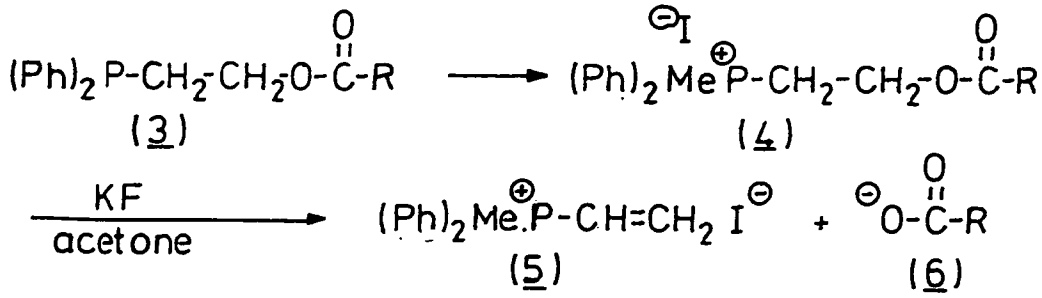


This series of compounds has been used extensively in mechanistic studies of β-elimination by Stirling (1.3.2)

1.2.2 Safety-catch principle

The elimination of the 2-thiomethylethyl group, *via* oxidation, is an example of a class of compounds which are completely stable under normal reaction conditions until activated either by quaternisation or by oxidation to produce a species which eliminates easily (safety-catch principle). Other examples of this principle have been supplied by the development of the thioethyl link as a 'handle' in solid phase peptide synthesis[14,15]. Removal of the peptide is mediated by oxidation of the thioether to the sulphone with a homogeneous mixture of 4N sodium hydroxide, methanol, dioxan (30:9:1). The 2-(phosphino)-ethyl group introduced by Kunz[16] for amine protection has proved highly acid stable and is efficiently removed with dimethylamine in methanol. In order to increase the general stability of this type of group, based on phosphorus, the 2-(diphenylphosohino)ethyl group (3) can be used. Removal is facilitated by activation with methyl iodide and elimination with potassium fluoride[17], Fig.1.4.

Fig.1.4

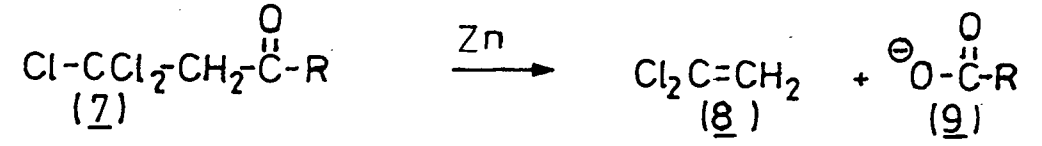


Katritzky and co-workers[18] have described the use of the 2- or 4-pyridyl ethyl group introduced by using 2- or 4-pyridyl ethanol or Michael addition of nitrogen, oxygen or sulphur nucleophiles onto the vinyl pyridine. Removal is obtained by activation with methyl iodide and elimination.

1.2.3 Reductive elimination

The first successful protection of a carboxyl function with a β-eliminating group was achieved by Woodward with the 1,1,1-trichloroethyl group for his synthesis of cephalosporin in 1966[19]. The principle used in this method is the same as used by Fischer[1], i.e. removal with zinc in acetic acid.

Fig.1.5



The use of the trichloroethyl group continues to be widespread in organic synthesis[19b,c]. A number of variations have been introduced by substitution of the chlorine with other halogens such as bromine[20,21] and iodine[22]. The 1,1,1-trichloroethoxycarbonyl group for amine protection, introduced by Windholz and Johnson[23], is also removed with zinc in dilute acetic acid[24].

1.2.4 β-Aryl functionalised

The Y-group in Fig.1.2 can be a substituted phenyl ring. Pfleiderer and co-workers have described examples of variation of the substituent group on the ring with NO_2, CN and Cl [25].

Fig.1.6

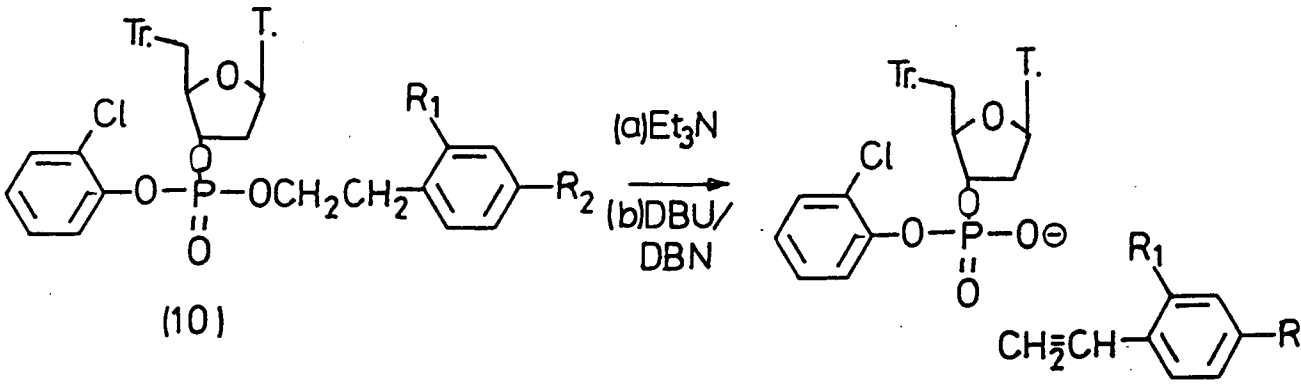
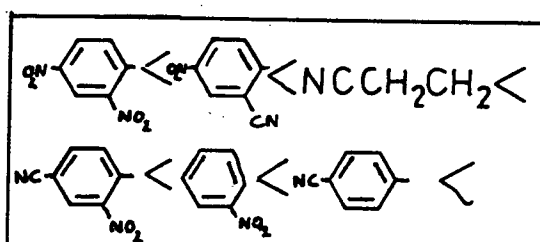
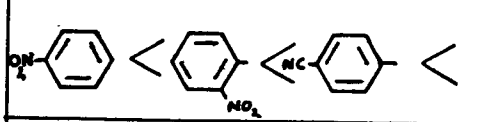


Table 1.1

	Et_3N
	DBU DBN
	stable

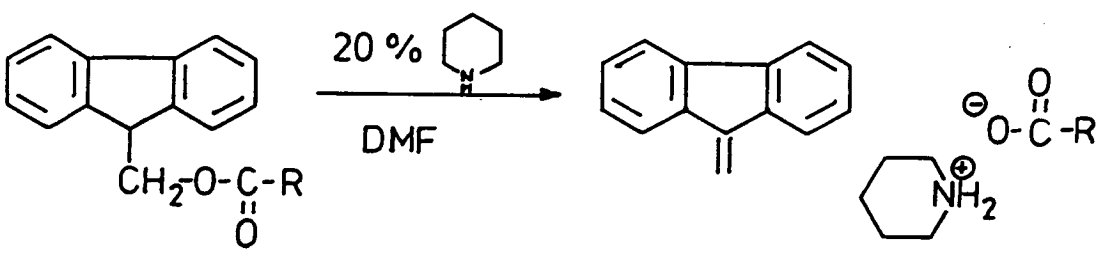
From this study Pfleiderer and Uhlmann[25] were able to choose the p-nitrophenylethyl group as having intermediate lability between the pyridine labile 2,4-dinitro phenylethyl group and the base stable phenylethyl group.

Deprotection of their new group was obtained with 0.5M DBU in pyridine[26]. More recently Pfleiderer and co-workers have used the 2,4-dinitrophenyl group in combination with the p-nitrophenyl group using a competitive deprotection scheme[27].

1.2.5 9-Fluorenylmethoxycarbonyl group

Introduced by Carpino and Han in 1971 for amine protection[28].

Fig.1.7



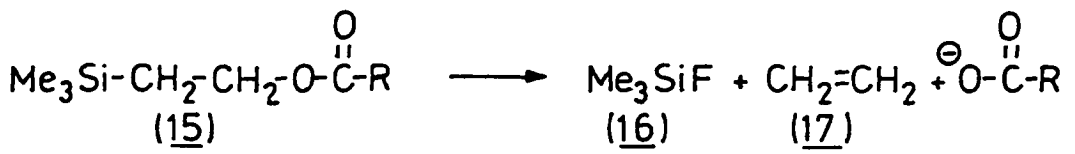
(13) (14)

Removal is accomplished with secondary amines such as 20% (v/v) piperidine in DMF and has found considerable use in solid phase peptide synthesis[29,92] (see Section 1.4.3) as well as for general carboxyl group protection[30].

1.2.6 Silicon derivatives

The trimethylsilylethoxycarbonyl group was introduced by L.A. Carpino in 1978[31] and is removed with tetra(alkyl) ammonium fluoride, strong acid or lewis acid. Elimination is also rapid with anhydrous TFA[32,33], see Fig.1.8.

Fig.1.8



(15) (16) (17)

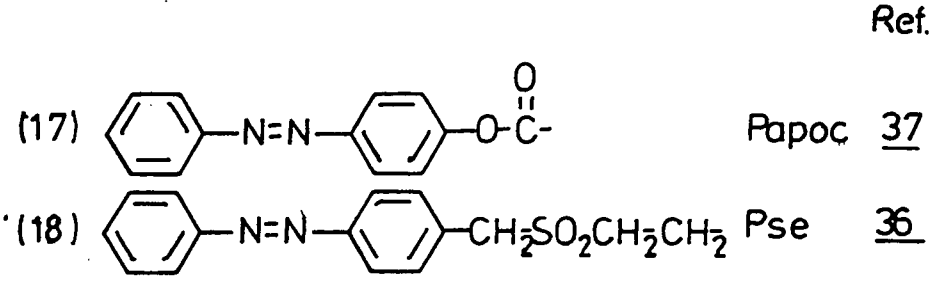
Deprotection of (*15*) with mild acid has the advantage that the by-products (*16*) and (*17*) are gaseous and can be removed *in vacuo*.

1.2.7 Other compounds

Wünsch and Spanenberg[34] introduced the cyano-t-butoxycarbonyl group which is removed with potassium carbonate. The β-cyanoethyl group[35] is commonly used for phosphite protection in oligo-nucleotide chemistry and is removed with triethylamine.

Groups derived from the 4-phenylazo moiety have been

Fig.1.9



introduced to provide a colorometric handle so that reactions or purification can be visually monitored. The Papoc group[37] can be removed with (1) alkali, (2) 4-dimethylaminopyridine in THF (2.8 g, in 40 ml, *ca.*10 sec at room temp.), (3) by transesterification with β-cyano ethanol/Et_3N/H_2O = 1:1:1 (1 min at room temp.), then elimination with diazabicycloundecene/pyridine (1 min at room temp.). The Pse group is removed with alkali[36].

1.2.8 1,6-Eliminating groups

The 5-benzisoxazolylmethyleneoxycarbonyl (Bic) group (*19*) was introduced in 1975 by Kemp and Hoyng[38] and is cleaved with triethylamine in DMF or acetonitrile whilst the

4-isopropyloxycarbonyloxybenzyloxycarbonyl[39] is cleaved with 0.1N NaOH to produce quinone methide (*25*) and the free amine (*24*), see Figs. 1.10 and 1.11.

Fig.1.10

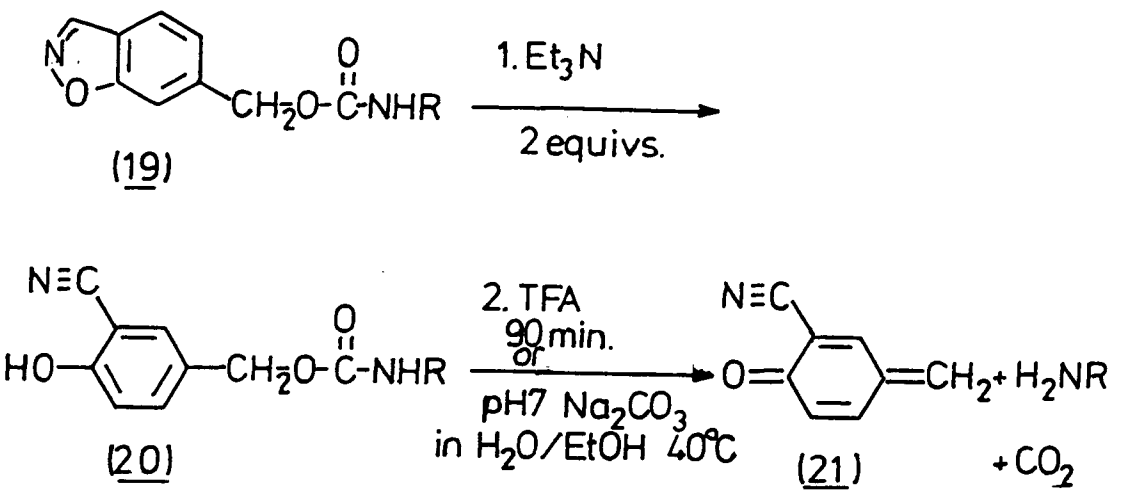
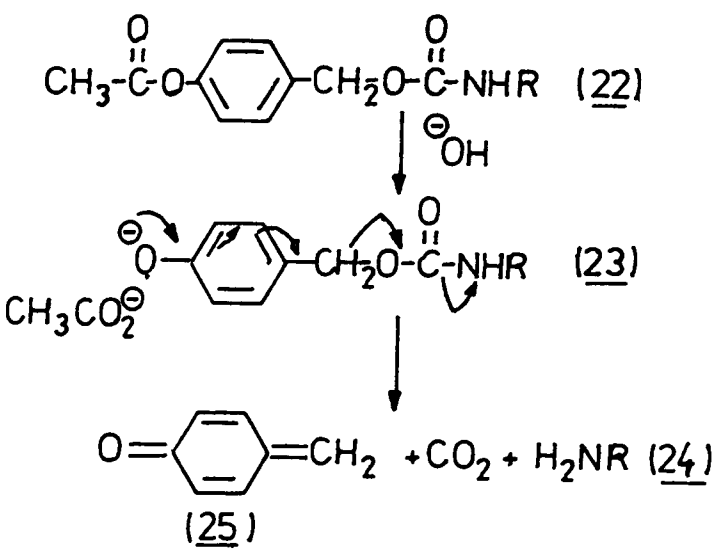


Fig.1.11



1.3 FACTORS AFFECTING β-ELIMINATION

To be able to design a novel β-eliminating group the basic physical chemistry underlying the concept must be understood.[40]

1.3.1 Acidity of β-CH bond

The first and most obvious requirement is the presence of an acidic proton on the β-carbon capable of abstraction with base. The acidity of the β-carbon protons is governed by four factors:

(i) hybridisation of the carbon

The pKa of the carbon acid is increased as the p-orbital contribution is increased effectively reducing the acidity as shown below[41-43].

Fig.1.12

	C_6H_5-C≡CH (sp)	CH_2=CH_2 (sp^2)	C_6H_{12} (sp^3)
pK_a	23	44	52

As the p-orbital contribution is increased then the electronegativity of the hybridised carbon is decreased due to the electrons on average being further removed from the nucleus with respect to sp hybridisation.

(ii) alkyl substituents on the carbon acid centre

The carbon acidity is reduced as the number and size of alkyl substituents is increased due to the destabilisation of the anion of the conjugate base[44,45].

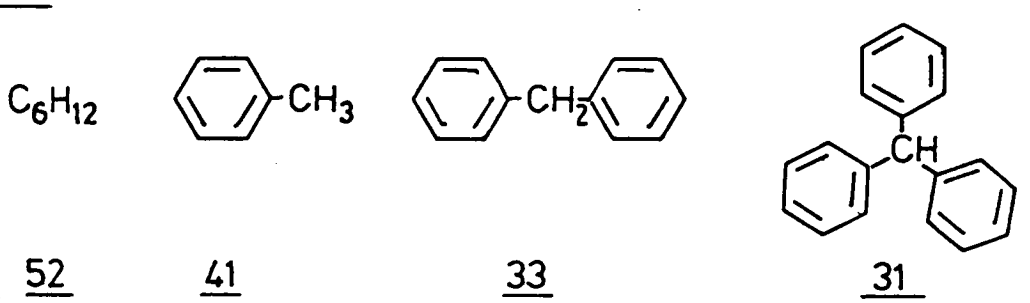
Fig.1.13

	CH_4	C_6H_{12}	$(CH_3)_3CH$
pK_a	48	52	71

(iii) conjugative stabilisation

Addition of groups on the β-carbon capable of delocalising the anionic charge has the effect of increasing the incipient acidity of the β-hydrogen, illustrated by the following series[46]:

Fig.1.14



pK_a	52	41	33	31

For maximum conjugative effect then the phenyl rings are required to be coplanar with the anionic centre. This is not possible for diphenylmethane due to the ortho hydrogens interfering sterically to twist the rings into a propeller conformation with perhaps some enlargement of the angle between the central carbon atom and the phenyl rings[47] (see appendix B). This distortion can be seen by comparison with fluorene in which the two aromatic rings are bonded together thereby removing the ortho hydrogen interference.

Fig.1.15

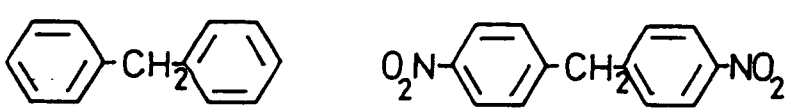
pK_a	23	33

Fig 1.14 also illustrates the cumulative effect of adding anion stabilising groups to an acidic centre. A saturation point is reached after which further conjugative stabilisation has relatively little effect on pKa[46].

(iv) electron-withdrawing group stabilisation

Of perhaps most importance is the effect of electron withdrawing groups on the β-carbon acidity. The series below[48], Fig 1.16, illustrates how on increasing the electron withdrawing power of the β-substituent the ionised carbon is stabilised through delocalisation into the electronegative centre.

Fig.1.16



pK_a	33	15.8

1.3.2 Role of the leaving group

The second important influence on the stability of a β-eliminating group is the leaving group. Should this species be changed to a particularly good leaving group then the stability of the molecule will be decreased and

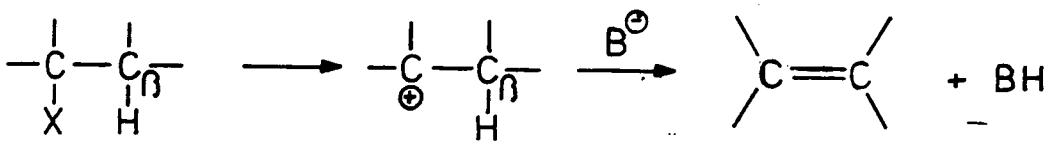
conversely so if it is substituted for a poorer leaving group. Stirling and co-workers have carried out extensive studies on β-phenylsulphonyl and β-cyanoethyl derivatives with a wide range of leaving groups[49,50]. From this work he was able to define nucleofugality[49,51-53] as a quantitative measure of a leaving group's ability to cleave with the bonding pair of electrons in the rate determining step. It was observed that leaving group ability does not correlate either with the pKa of the conjugate acid of the leaving group, or with the leaving group nucleophilicity. However this area is still in the early stages of development so that a leaving group's ability is usually referred to in a relative or qualitative measure.

From the method of elimination there can be obtained useful information such as the influence of solvent, base, temperature and effect of changing the protected species on elimination. In general, elimination reactions can be classified into three main categories:- E1, E1 conjugate base and E2.

E1

The E1 mechanism involves the loss of X, a leaving group, to produce a positive centre which affords the double bond *via* abstraction of the β-proton.

Equation 1.1

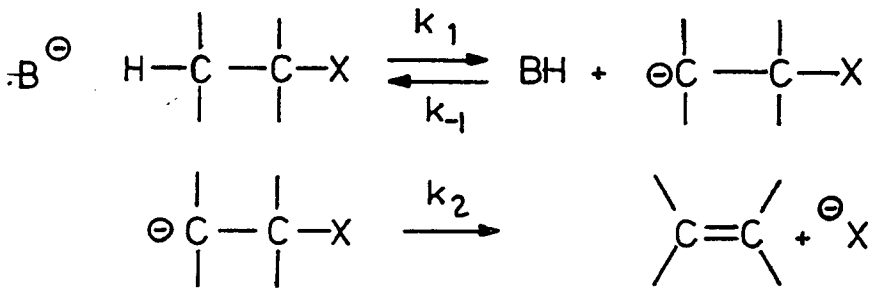


This mechanism is favoured when the substrate can form a relatively stable carbo cation, possesses a good leaving group and when an ionizing solvent of low nucleophilicity is used. An example of an E1 mechanism is given by the elimination of tertiary and secondary tosylates or halides in acetic acid[54].

E1cB

The second type of elimination involves the abstraction of the β-proton to leave a carbanion centre which decomposes to give the double bond with loss of the leaving group.

Equation 1.2

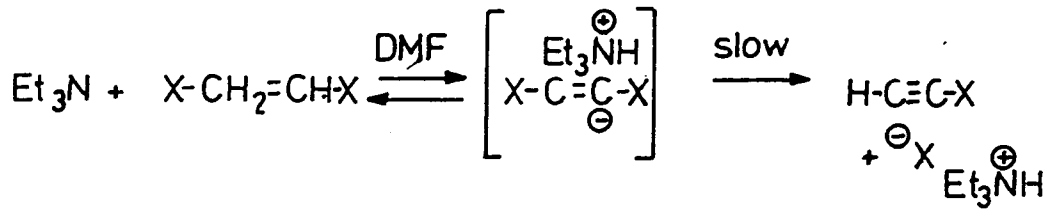


Within the general category of E1 carbanion (known as E1cB) there are four types of mechanism differing with the varying degrees of timing of H-C and C-X bond breaking. The first type is $(E1cB)_{IRR}$ when $k_2 >> k_1, k_{-1}$, see eqn.1.2, i.e. the leaving group is so good that proton abstraction becomes the rate determining step. Reactions of this type are dependent on base and substrate concentration, giving overall second order kinetics[55].

The second and third type of mechanism $(E1cB)_{IP}$ and $(E1cB)_R$ are closely related. These mechanisms occur if the β-hydrogen atom is made more acidic whilst the leaving group is made poorer, thus allowing the reversible formation of the carbanion i.e. $k_1, k_{-1} > k_2$ (eqn.1.2). $(E1cB)_{IP}$ involves

the reversible formation of a closely related ion-pair whereas in the $(E1cB)_R$ mechanism the intermediate species are distinct. The $(E1cB)_R$ mechanism would be expected to be a specific (or thermodynamical) base catalysed elimination.

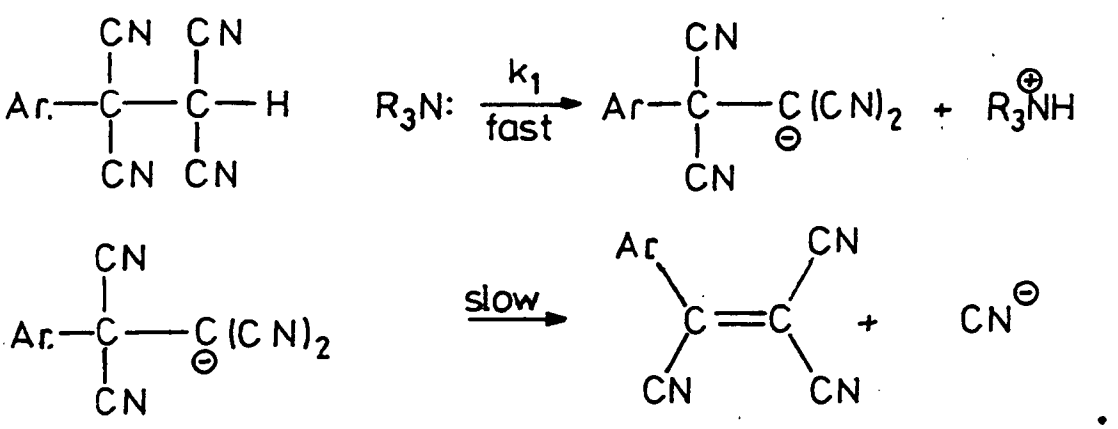
Equation 1.3



In the $(E1cB)_{IP}$ and $(E1cB)_R$ mechanisms no retardation by BH^+ would be expected and the reaction would be second order influenced by change in pH only.

The final type of elimination is the (E1) anion in which the β-hydrogen is made even more labile and the leaving group very poor, it is then possible to obtain a situation where $k_1 > k_{-1} >> k_2$. An example of this type of mechanism[56] is shown in equation (1.4).

Equation 1.4



If more than an equimolar amount of base is present then rate is independent of base concentration. However

in this example an equimolar quantity of base is not required since HCN is such a weak acid that free base is continually reformed as shown in eqn.1.5.

Equation 1.5

$$R_3NH^{\oplus} + CN^{\ominus} \rightleftharpoons R_3N: + HCN$$

This type of elimination is relatively rare due to the extreme acidity of the β-hydrogen required. The main features of an (E1) anion mechanism are overall first order kinetics, a primary deuterium isotope effect of 1 and rate of elimination would be expected to increase with electron-releasing substituents on the β-carbon.

Effect of solvent

For a two step process such as ElcB reactions, the effect of solvent change will influence the elimination in four ways:[57] (i) solvation of the substrate, (ii) solvation of the carbanion, (iii) solvent effect on elimination from the carbanion, i.e. on k_2, (iv) solvent effect on reprotonation of the carbanion. For uncharged substrates the effect of changing solvent is relatively small ($\sim$ factor of 10 is the largest observed in H_2O/ethanol system) so that discussion of the effect of solvent change in organic solvents may be expected to be smaller than a factor of 10. If one considers a relatively non-polar and polar solvent system (CH_2Cl_2 and DMF) then the carbanion produced in the pre-equilibrium mechanism will be better solvated in a polar solvent, thereby favouring formation. Release of the acid from the carbanion

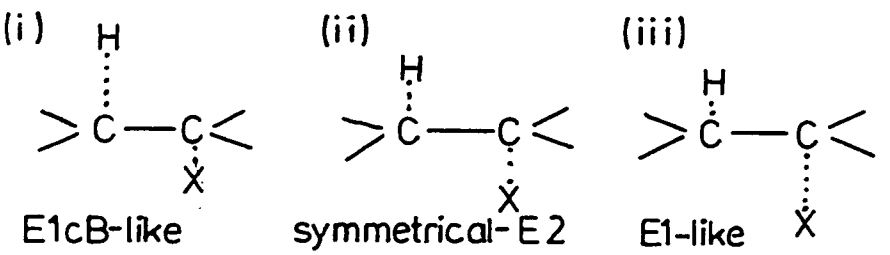
intermediate will also be favoured in a relatively polar solvent.

Determination of the type of mechanism for elimination reactions has been investigated using kinetic order, β-proton exchange with respect to rate of elimination $(ElcB)_{IRR}$ versus $(ElcB)_{R}$, general or specific base catalysis $(ElcB)_{R}$, isotope effect on rate or substitution on the β-carbon with electron-donating or withdrawing groups and leaving group isotope effect[55].

E2

The third class of elimination reactions is the E2 mechanism. Pure E2 describes a concerted elimination process where C-X and C-H bond breaking are involved in a symmetrical transition state. However it is thought that there exists a variation of transition states[58,59] ranging from one similar to ElcB, in which C-X bond breaking is preceded by C-H bond breaking (i), to one similar to the E1 mechanism so that the C-X bond is elongated with respect to C-H in the transition state of the rate determining step. Intermediate to these extremes is the symmetrical transition state (ii) see eqn.1.6.

Equation 1.6



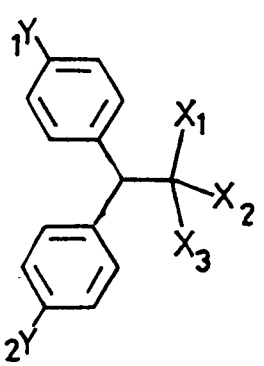
A reaction proceeds via an E2 mechanism if the β-hydrogen is partially but not completely transferred and the C-X bond, partially but not completely, broken in the transition state of the rate-determining step. These types

of elimination should exhibit second order kinetics, have a primary isotope effect for the β-proton and be dependent on the nature of the leaving group.

As several E1cB mechanisms are second order, differentiation between E1cB and E2 can be difficult. Experimental observation of the respective theoretical kinetic properties can be confusing as these mechanisms exhibit one or more characteristic features.

An example of the great interest in this area can be illustrated by the use made of 2,2-diarylethyl halides. shown in Fig.1.17.

Fig.1.17



$Y_1 = Y_2 = H$; $X_1 = X_2 = X_3 = Cl$ (26)

$Y_1 = Y_2 = X_1 = X_2 = Cl$, $X_3 = H$ (27)

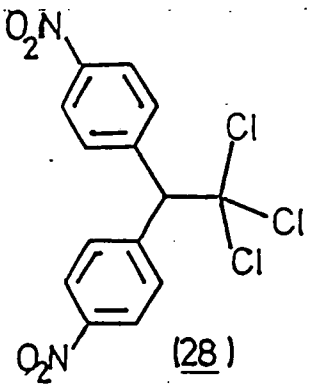
$Y_1 = Y_2 = NO_2$

(a) $X_1 = X_2 = X_3 = Cl$ (28)

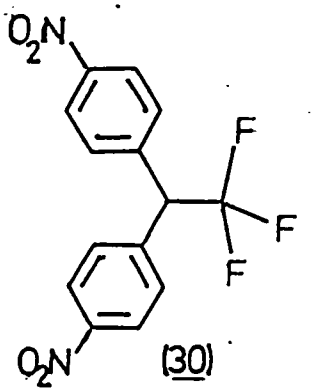
(b) $X_1 = X_2 = Cl$, $X_3 = H$ (29)

A very large number of aryl substituent/leaving group combinations have been investigated. The advantage of their use is that subtle changes can be effected by the variation of the substituent/leaving group combination which can lead to traversing across the E2-E1cB spectrum of mechanisms, effectively changing the ease of formation and stability of the anion. McLennan and co-workers[60-64] described the elimination of 2,2-bis(4-nitrophenyl)-1,1,1-trichloroethane (*28*) to be $(E1cB)_{IP}$. This research group then searched for an example of a border line E1cB-E2 (or limiting E1cB) mechanism. This was thought to have been found with 2,2-bis(4-nitrophenyl)-1,1-dichloroethane (*29*)[63].

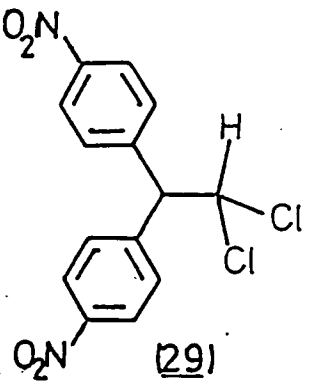
Fig.1.18



(28)



(30)



(29)

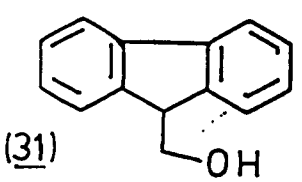
pK_a 11.8 | 11.3 | 16.5

$[E1cb]_{IP}$ | $[E1cb]_R$ [67] | E1cb-limit or E1cb-E2

However recent work by Fry and Pulay[65] using α-carbon isotope effect rather than chlorine isotope effect clearly shows that bonding changes do occur at the α-carbon in the rate determining step, suggesting an E2 mechanism.

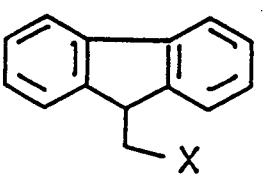
A final example of how leaving group changes affect the mechanism of elimination is shown below.[66]

Fig.1.19



(31)

E1cb



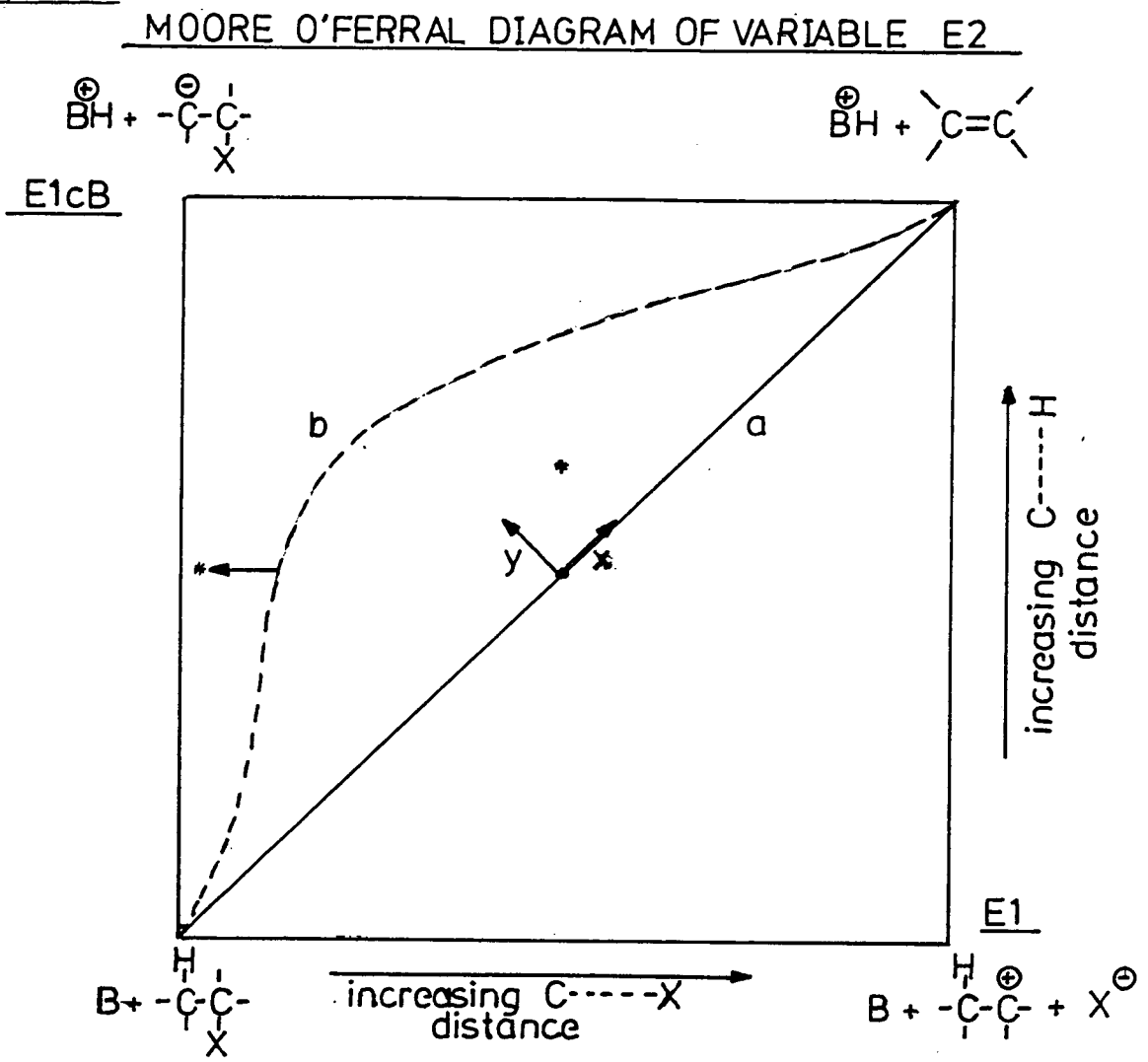
E2

X=Cl or Br

(32) (33)

When the hydroxy group in (*31*) is replaced with better leaving groups (e.g. X=Br (*33*) Cl (*32*)) the mechanism has been found to change from E1cB to E2.

Fig.1.20



The Moore O'Ferrall potential energy diagram provides[66b,66c] a convenient framework for summarising the effect on the transition state of the factors discussed. If a central E2-type transition state is considered then the reaction path for this mechanism can be given as a straight diagonal across the diagram; the two components contributing to this path being symmetric. If a change to a poorer leaving group is considered then this would result in an increase in energy of the E1 ion pair intermediate moving the transition state upwards on the diagram (along the reaction path vector x and perpendicular vector y). The net result would be a new transition state with an increased carbanion character.

The approximate reaction coordinates for an E1cB-like transition state is indicated by the dashed lines (b). A change to a poorer leaving group will cause a transition state with less C_{α}-X bond breaking, more carbanion character and little change in the C_{β}-H bond breaking.

1.4 PEPTIDE CHEMISTRY

1.4.1 Introduction

Peptide chemistry for many is the apotheosis of the use of protecting groups as a synthetic tool. In essence peptide synthesis ideally requires four different types of protecting groups.

(1) Nα protection of one component

(2) Cα protection of the other component

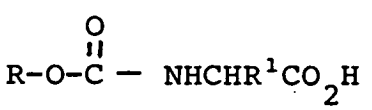
(3) Cysteine thiol protection

(4) Other side-group protection

A further requirement is for at least one of these classes of groups (normally Nα) to be removed in the presence of the remaining groups. This can be achieved in a number of ways depending on either the resistance of the group to acid (graded acidolysis) or by employing chemically independent removal conditions (orthogonality). Both of these types of protecting group strategy are used in peptide synthesis and will be discussed later.

1.4.2 (1) N α Protection - urethane groups

The introduction of the benzyloxycarbonyl group[68] (removed with hydrobromic acid in acetic acid or catalytic hydrogenation) provided a prototype from which many other successful urethane-type protecting groups have been derived having the general form:



where the nature of R determines the method of cleavage.

Fig. 1-21

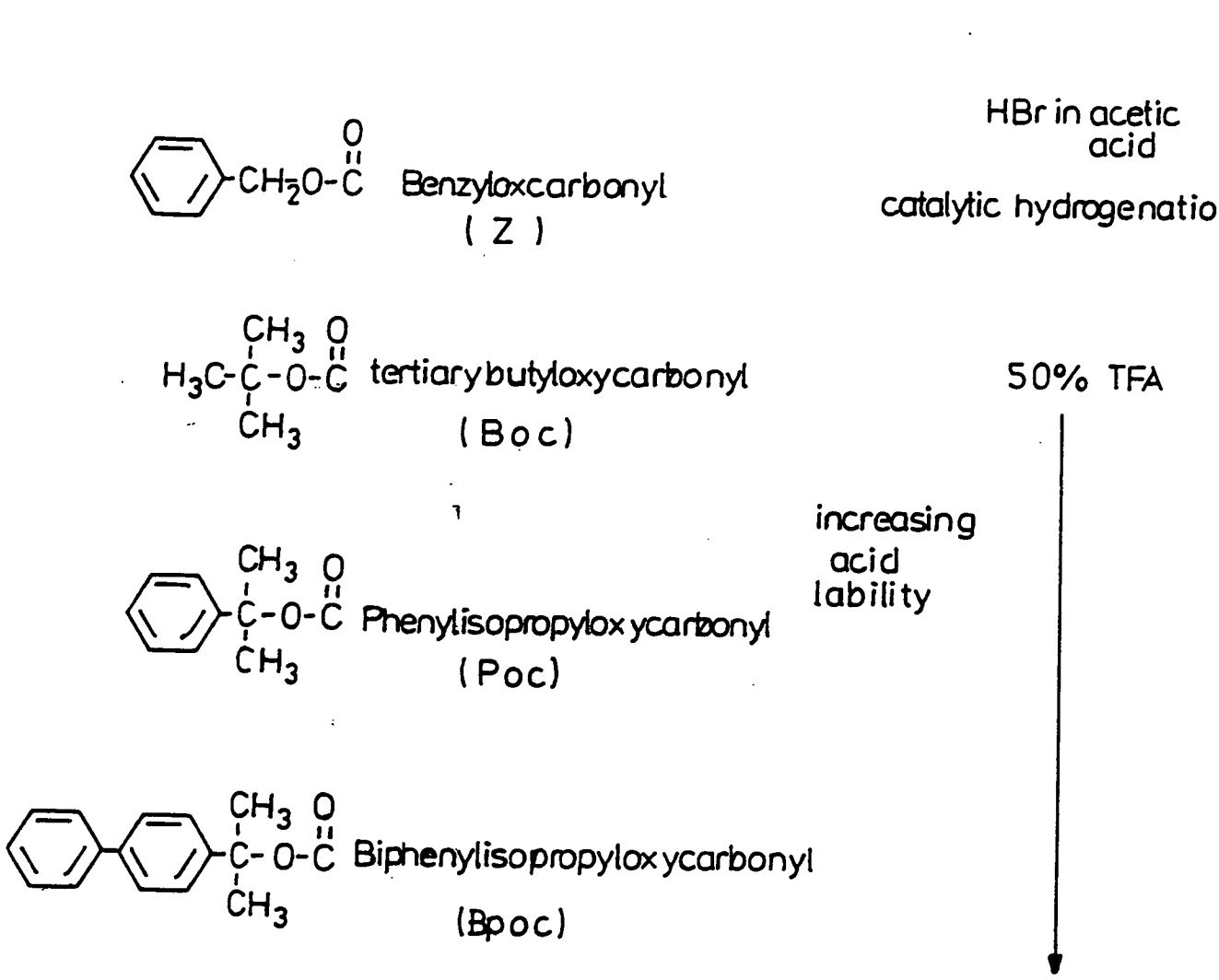
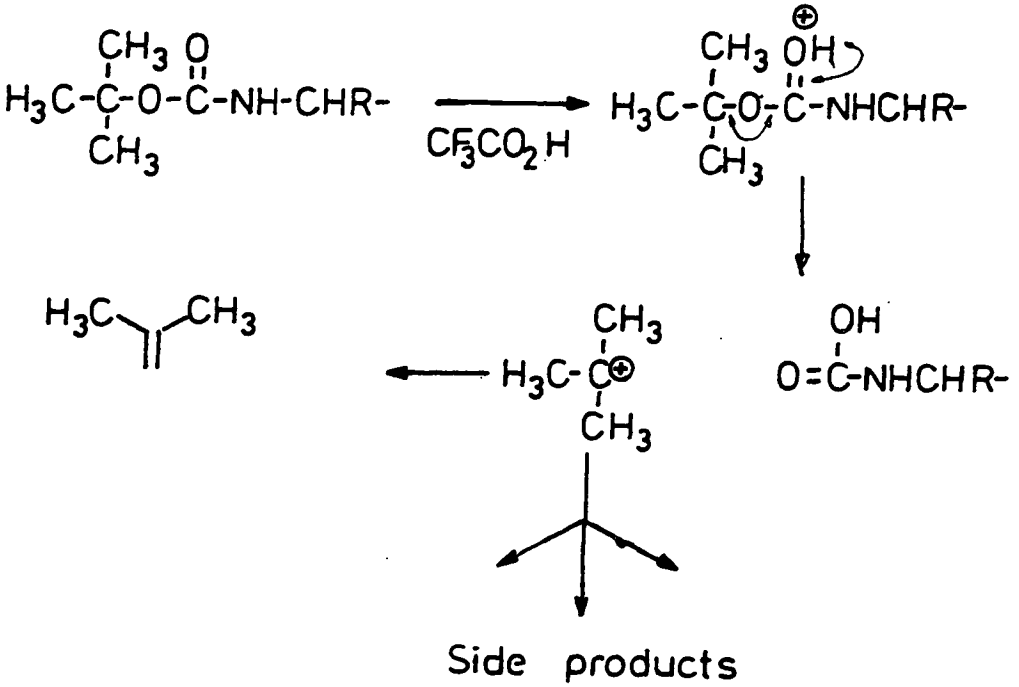


Fig. 1-22



Acidolysis

The t-butyloxycarbonyl group[69] has proved the most successful and durable of this class. Removal with 50% TFA in dichloromethane, together with good stability to base confers excellent characteristics. Combination with the benzyloxycarbonyl group for side-chain protection allows selective Nα deprotection. Substitution of a methyl group in Boc for biphenyl (Bpoc)[70] or phenyl (Ppoc)[71] has provided a family of acid sensitive groups with graded degrees of lability towards TFA[71] (see Fig.1.21). A major disadvantage of using Boc-type protecting groups is the generation of reactive carbonium ion intermediates on deprotection. As will be illustrated this necessitates great care to be taken over deprotection conditions and restricts the use of Boc in association with the amino acids (tryptophan, tyrosine, cysteine and methionine) having side chain functionality reactive towards carbonium ions.

The generation of tertiary alkyl carbanion ions during acidolysis with TFA was predicted to produce trifluoroacetates by the work of Weygard and Steglich[72]. Experimental evidence provided by Lundt and co-workers[73] proved these species to be highly reactive alkylating agents. Mono-or multi-alkylation on the indole ring of tryptophan to as much as 14% has been recorded by numerous workers[74-77]. Tyrosine can be alkylated either on the phenolic hydroxyl group or at the 3 position[73]. Protection of the tyrosine hydroxyl group as a benzyl ether is known to cause an acid catalysed 'benzyl-migration'[78] (for example, see Fig.1.23). C-Alkylation can be reduced by deprotection with TFA/water

Fig.1.23

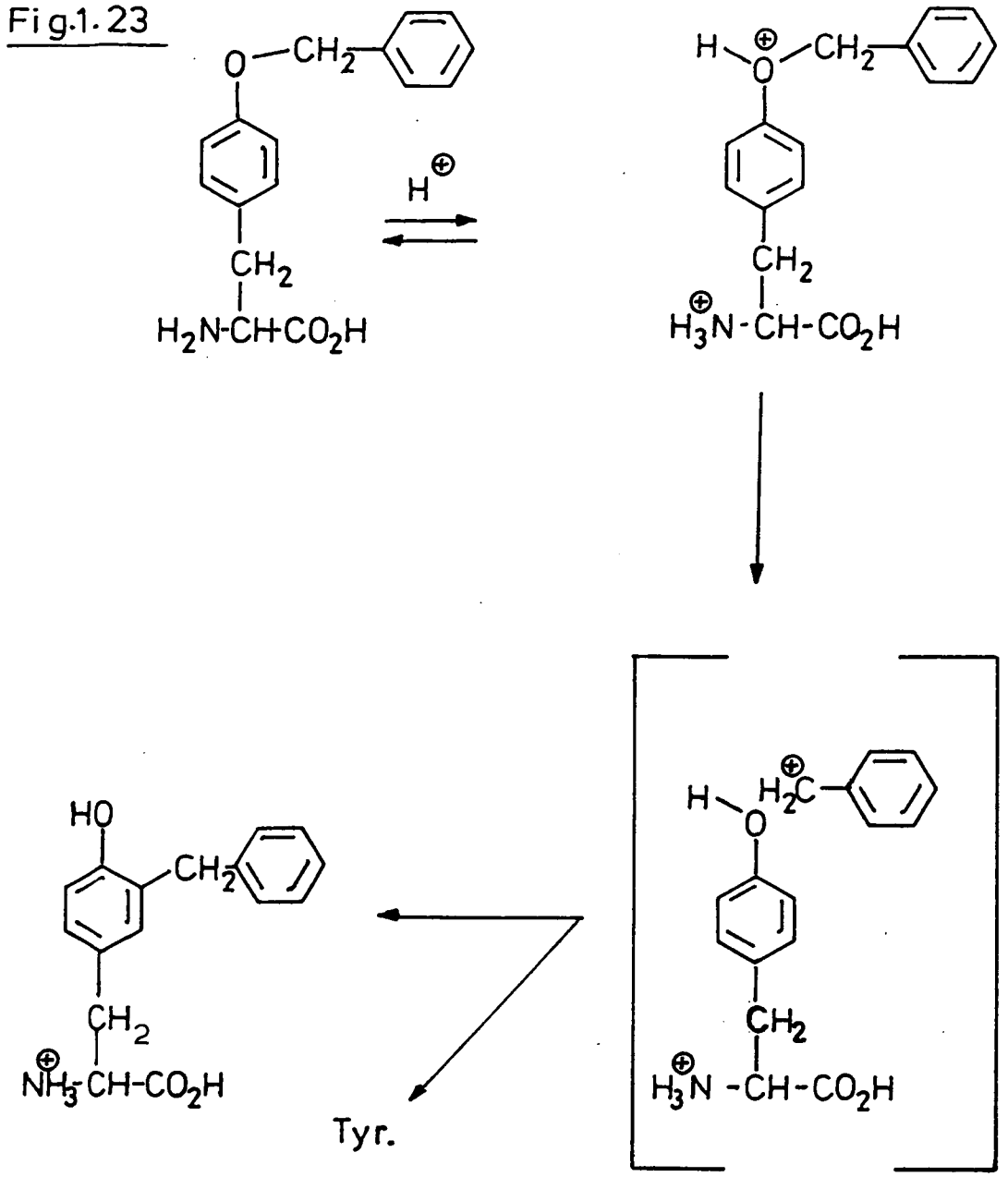
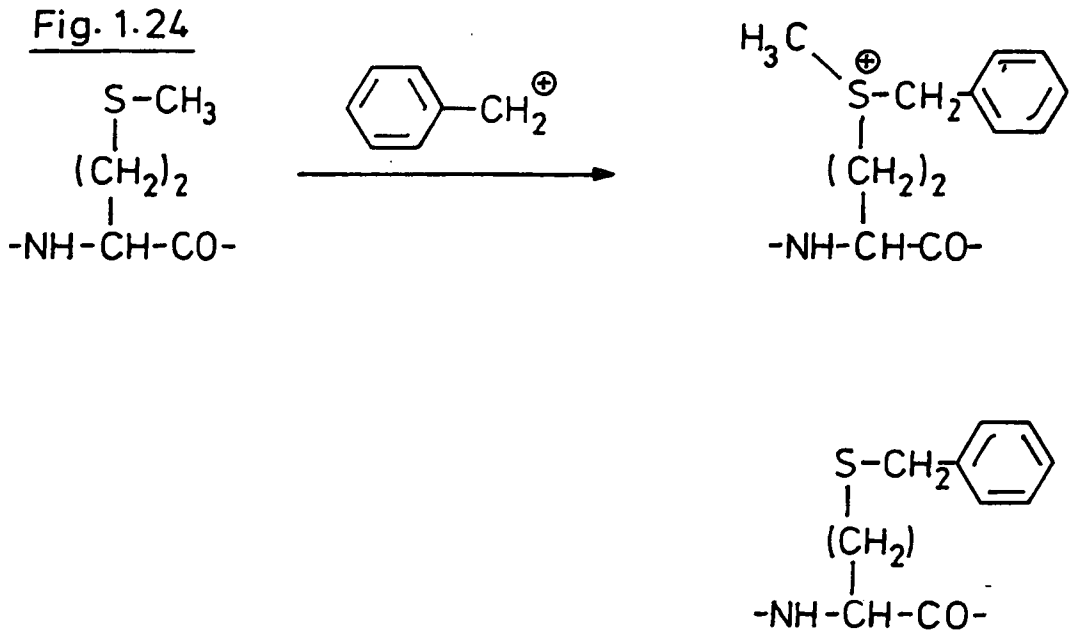


Fig. 1.24



or acetic acid. The presence of scavengers such as anisole thioanisole or 1,2-dithioethane-dimethylsulphide, can reduce, but not remove, alkylation leading to speculation about the 'benzyl migration' mechanism. The presence of alkylating agents necessitates the protection of hydroxyl groups (serine and threonine) and the carboxylate groups of aspartic and glutamic acid.

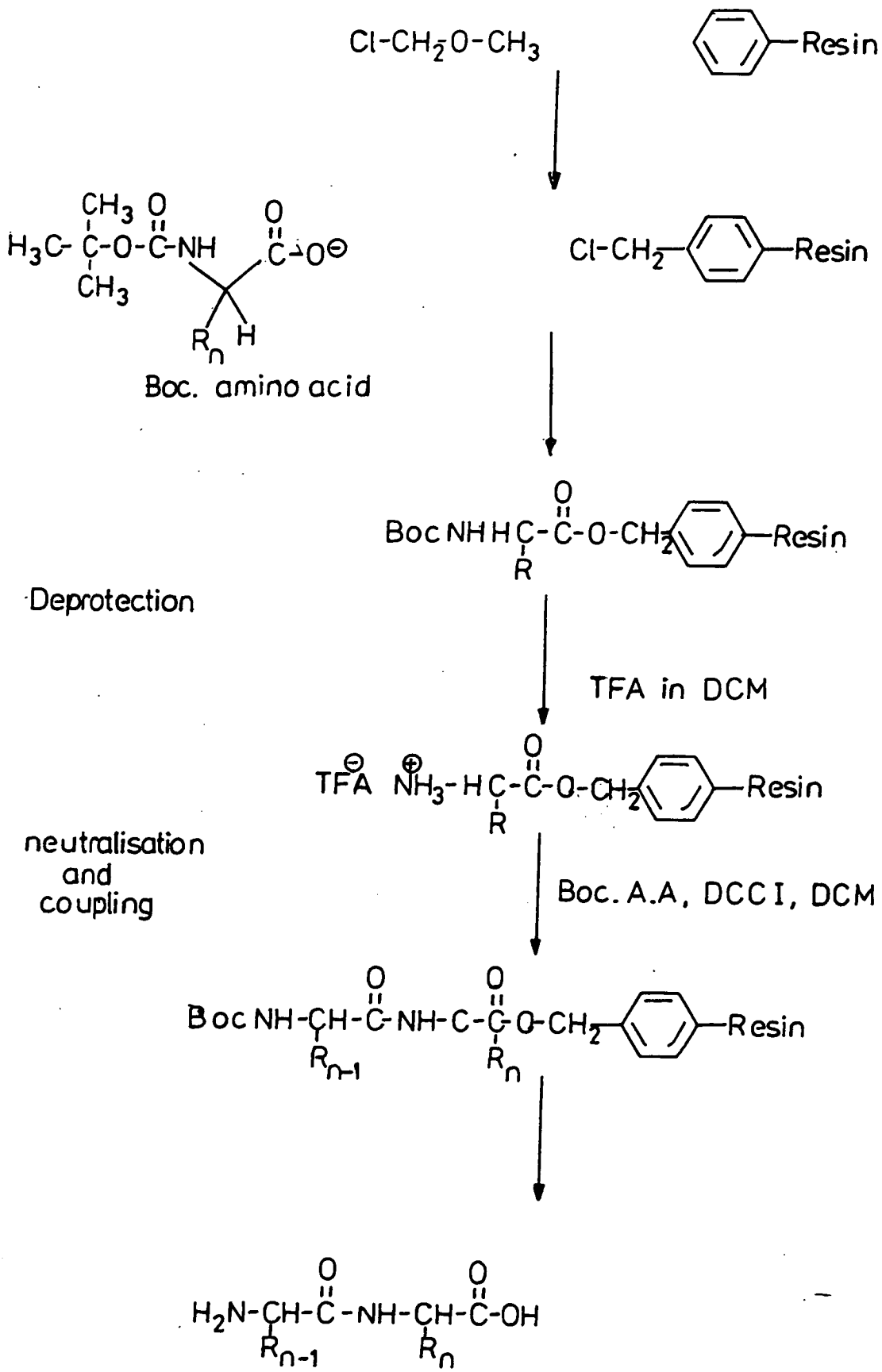
Removal of the benzyloxycarbonyl group with HBr/acetic acid in the presence of either cysteine or methionine leads to the formation of S-benzyl salts which decompose to give S-benzylhomocysteine[79,80] rather than reverting back to methionine (see Fig.1.24). Cleavage by catalytic hydrogenation requires extended reaction times as sulphur containing compounds have been found to poison the catalyst. Successful removal of the Z-group in the presence of methionine has been achieved using liquid ammonia as the solvent[81].

An alternative side-chain protection to benzyloxycarbonyl is provided by the diphenylphosphinyl group[82]. Deprotection with hydrogen chloride in methanol produces the inert diphenylphosphinyl methyl ester thereby avoiding any reactive intermediates.

1.4.3 Solid phase peptide synthesis

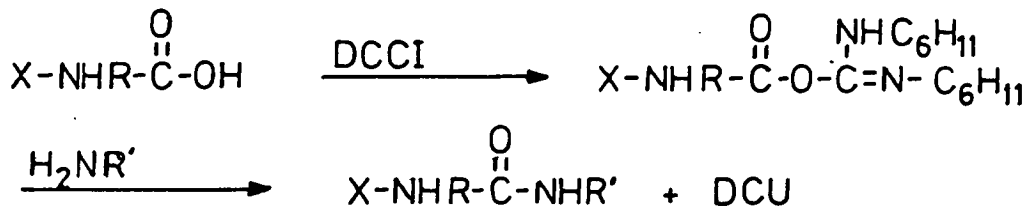
In 1963 Merrifield introduced the concept of peptide synthesis on an insoluble polymeric support[83,84]. The resin used was polystyrene crosslinked with divinylbenzene. The best practical results were obtained with 1% divinylbenzene which provided a mechanically robust resin capable of filtration and mixing coupled with good swelling characteristics in dimethylformamide or dichloromethane. Resins with

Fig. 1.25


Cl-CH2-O-CH3
Resin
Cl-CH2
Resin
Boc. amino acid
Boc NH H C-C-O-CH2
Resin
Deprotection
TFA in DCM
TFA⊖ NH3⊕-H C-C-O-CH2
Resin
neutralisation and coupling
Boc. A.A, DCC I, DCM
Boc NH-CH-C-NH-C-C-O-CH2
Resin
H2N-CH-C-NH-CH-C-OH

greater than 1% cross-linking were found to have good mechanical properties but poor solvent and reagent penetration, whilst resins with less than 1% were found to be too fragile. The cesium salt of the first Nα-Boc protected amino acid was reacted with a chloromethylated resin (see Fig.1.25). The Boc-protecting group was removed and the TFA salt produced neutralised with triethylamine. The next protected amino acid was then activated either through its symmetrical anhydride or with dicyclohexylcarbodiimide.

Fig.1.26



The acylating reagent is normally used in a four-fold excess to ensure complete reaction. Removal of reagents by filtration was followed by cleavage of the Boc-group and the process repeated until the required sequence was obtained. The advantages of solid phase synthesis are:

(1) it avoids the losses normally associated with isolation and purification of all intermediates;

(2) individual reactions can be forced to completion by using excess reagents, removed by washing the polymer;

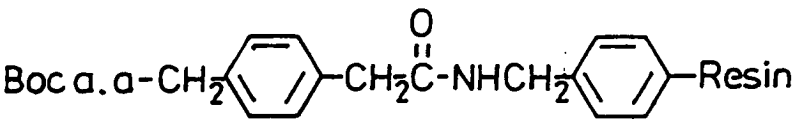
(3) the method lends itself to automation;

(4) transfer losses are removed as a single vessel is used throughout the synthesis.

The original scheme (Fig.1.25) introduced by Merrifield utilized a graded acidolysis strategy similar to that described for solution phase synthesis. The repetitive

use of TFA to remove the Boc groups was found to cleave the ester bound to the resin by about 1% per cycle leading to low yields of final product[85]. To overcome this problem the phenylacetamidomethyl link[86] between the ester and the resin was introduced to increase the acid stability by about 100-fold. Stability towards TFA can be explained by the electron withdrawing nature of the acetamido group in the para position of the phenyl ring destabilising the developing carbonium ion,although this has little affect against S_N2 acidolysis by HF.

Fig.1.27

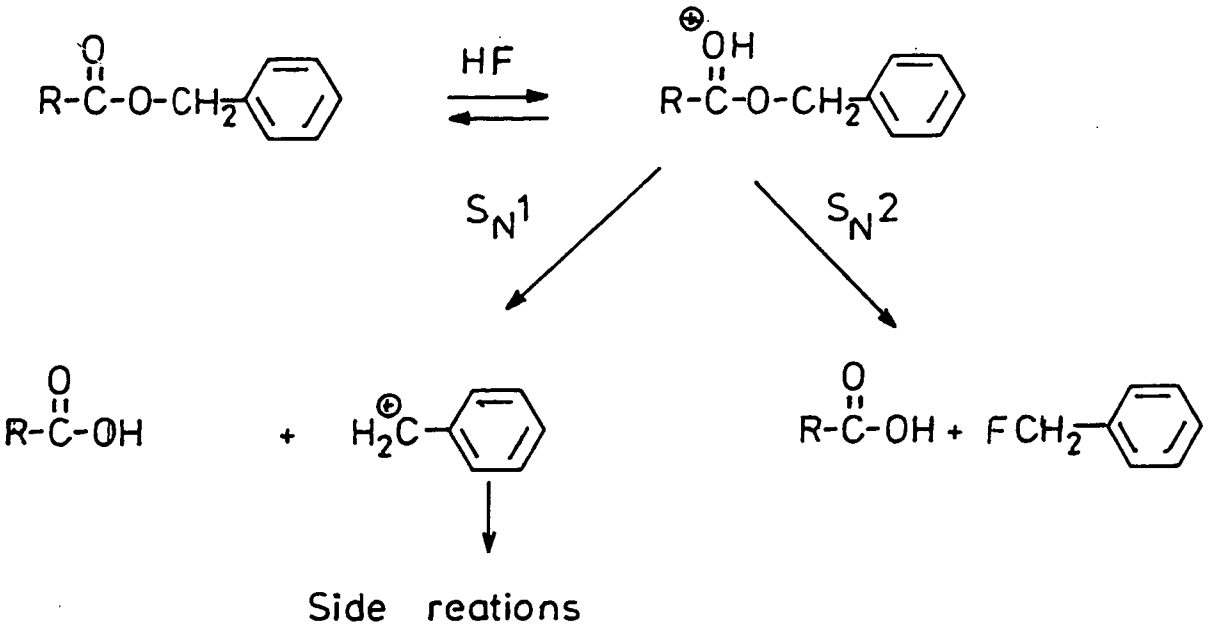


The ester link is cleaved with anhydrous HF. Simultaneous cleavage of all side chain protection liberates reactive carbonium ions mentioned earlier necessitating the use of scavengers. However HF was found to cleave the methyl ether link of anisole to produce a reactive intermediate known to alkylate the NH of tryptophan[77] as well as other acid sensitive residues. HF treatment can produce reactive acylating reagents from protonation and dehydration of glutamic acid and has been found to acylate anisole and other scavengers[87].

Great effort has been made to reduce these side reactions to a minimum and notable success was obtained with the introduction of HF/DMS (low-high). In this method it was found that by adjusting the concentration of HF in DMS to less than 55% the deprotection of O-benzyltyrosine went

through a change from S_N1 to S_N2 mechanism.

Fig.1.28



At higher concentrations of DMS the S_N1 mechanism is predominant leading to the generation of carbonium ions and side reactions. A combination of these conditions has been used to remove the precursors of the harmful carbonium ions at HF concentration below 55% and so by S_N2 mechanism whilst the more acid resistant anchoring bonds removed in the final step by strong acid (S_N1)[88].

However a protection scheme relying on differential kinetic stability of protecting groups can never be absolute and will inevitably lead to impurities. Even if the side products are only produced in very small amounts per cycle the cumulative effect over many cycles will lead to a serious lowering of yield and complicate the purification of the final product.

1.4.4 Chemically-selective protection strategy

The cleavage conditions for β-eliminating N α protection groups appear to offer considerable advantages. Firstly, the mild conditions of removal would reduce the number of side-reactions inherent in an acid-sensitive scheme. Secondly the possibility of using acid-labile side-chains and ester linkages would introduce selective deprotection conditions chemically independent from one another (orthogonal protection). In independent surveys of β-eliminating groups Meienhofer[89] and co-workers, as well as Sheppard[29] and co-workers concluded that the 9-fluorenylmethoxycarbonyl (Fmoc) group[28] was the most attractive possibility. The other protecting groups were discounted mainly due to the unacceptable use of strong alkali for their removal known to incur the risk of secondary reactions (racemisation, transpeptidation or degradation)[90,91]. In the case of β-ar(alk)ylsulphonyl derivatives by-products are possible due to reactive olefinic compounds produced on deprotection.

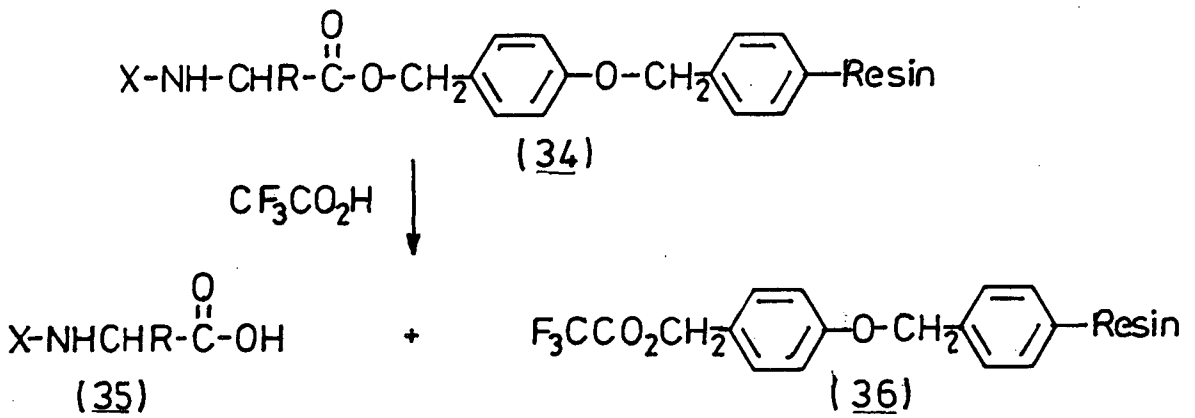
The base labile Fmoc group in combination with acid-labile side-chain protection and resin linker has provided the basic strategy from which further advances in orthogonal protection have been made.

Fmoc, t-butyl ester and p-alkoxybenzyl ester strategy

The Fmoc group and acid-sensitive t-butyl ester side chain protection[92] have been used with great success in combination with the acid labile p-alkoxybenzyl alcohol resin introduced by Wang[93]. The resin link can be cleaved with mild TFA by protonation of the carboxyl group followed by

transesterification. The electron donating ability of the para alkoxy group stabilises formation of a benzylic carbonium ion and so enables facile cleavage. The combination of mild base removal of Nα-protection and mild acid cleavage from the resin allow this strategy to avoid the destructive use of HF in the final deprotection step.

Fig.1.29



Although contact to acid, and hence to possible alkylating species has been reduced to a minimum, care must be taken at the final step if acid-sensitive residues are present. The development of this stragegy has led to the successful synthesis of many biologically active peptides. Some examples include substance P, A.C. protein (65-74)[94], human β-endorphin[95] and little gastrin[96].

Sheppard and co-workers have introduced a polyamide (polydimethylacrylamide) support for their syntheses. This resin has been shown to have excellent swelling characteristics in polar solvents (such as DMF) so allowing optimum conditions for deprotection and coupling. The latter feature is

attributed to the polar nature of the resin (relative to polystyrene) having similar physical properties to the growing peptide and presenting a more chemically uniform environment to the reagents. The usefulness of this approach was illustrated by the successful synthesis of A.C. protein (65-74)[94], a sequence found to be particularly difficult on a polystyrene support.

The Fmoc, t-butyl, alkoxybenzyl ester methodology appears to offer an excellent strategy for peptide synthesis combining mild conditions with efficiency. However it is thought that the repeated use of vast excesses of 20% piperidine solutions to remove the Fmoc-group could cause cleavage of the ester-linker bond during synthesis. The lability of the Fmoc group towards relatively mild reagents such as diisopropylethylamine[97] has also been noted. Attachment of the first amino acid residue on to the polymer support is usually accomplished with the symmetrical anhydride of the amino acid and about 0.1 equivalent 4-dimethylaminopyridine (DMAP) (see Section 2.5). However due to the lability of Fmoc in the presence of DMAP an extra sequence has to be introduced to the solid phase cycle so that a base-stable Boc protected amino acid can be used for attachment of the first residue.

The success of a solid phase synthesis, involving as it does so many chemical reactions, requires that even the smallest detail needs attention. The need for pure reagents such as amino acids (see Section 2.2.4) and solvents (Section 2.3.4) is essential as is the requirement for mild removal conditions and feedback information on these

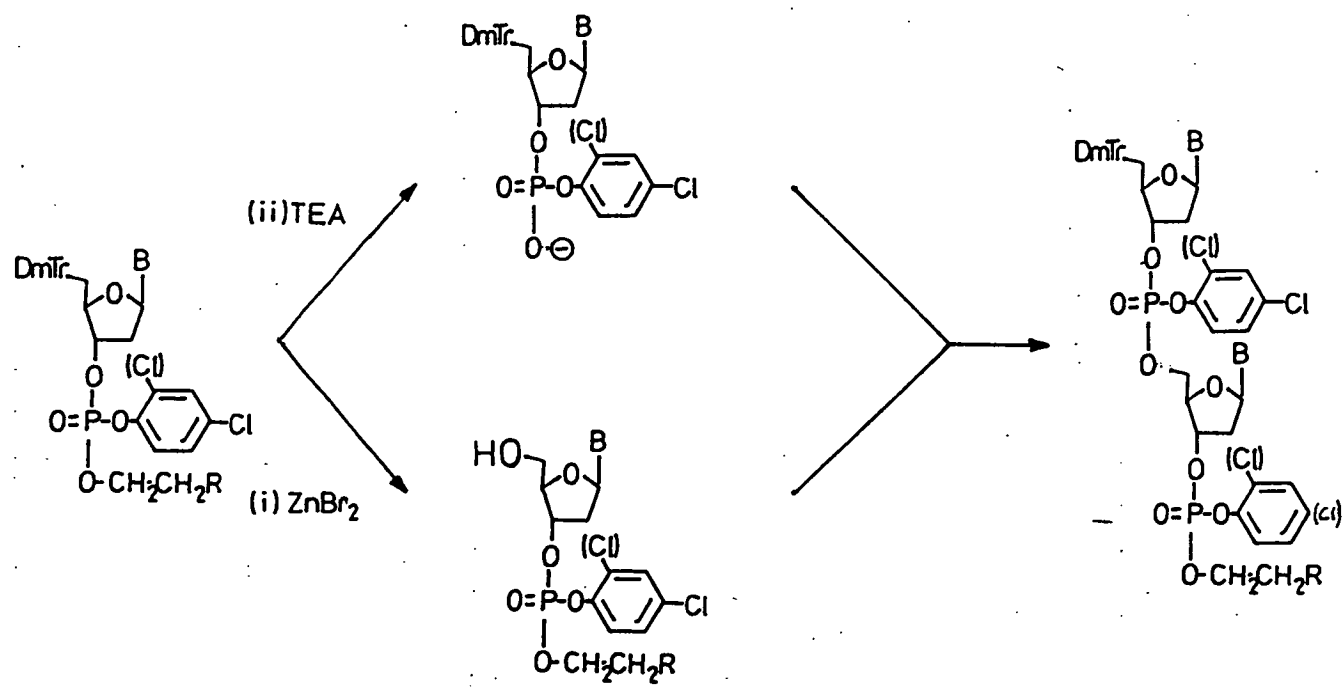
reactions. Finally a fast and efficient coupling between the respective amino acids is required to ensure high yield of the desired sequence.

1.5 Nucleotide Chemistry

Nucleotide chemistry and peptide chemistry share many similar problems such as dealing with highly sensitive, multifunctional molecules. The response to this challenge led to the phosphodiester method of synthesis developed by the pioneering work of Khorana[98]. Later improvements based on this method produced the phosphotriester followed by the modified phosphotriester approach[99]. The latter methods differ from the diester in that both dissociable phosphate functions are masked, largely ensuring the removal of problems associated with the solubility of the polar diester intermediates. This facilitates ease of purification and avoids possible condensation during coupling onto the phosphate backbone.

The phosphotriester approach has relied considerably on orthogonal protection, as shown in Scheme 1.

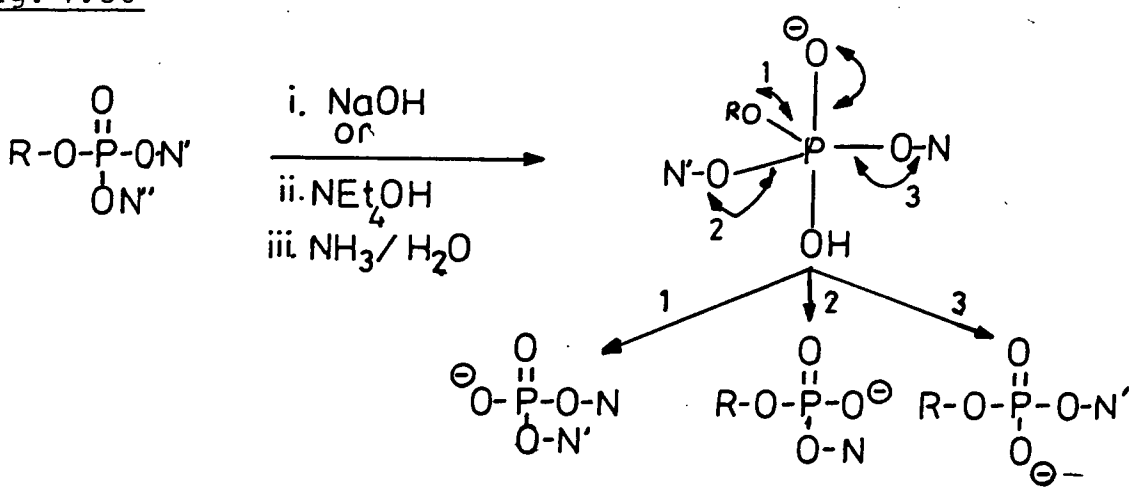
Scheme 1



DMTr -: dimethoxytrityl, R -: CN

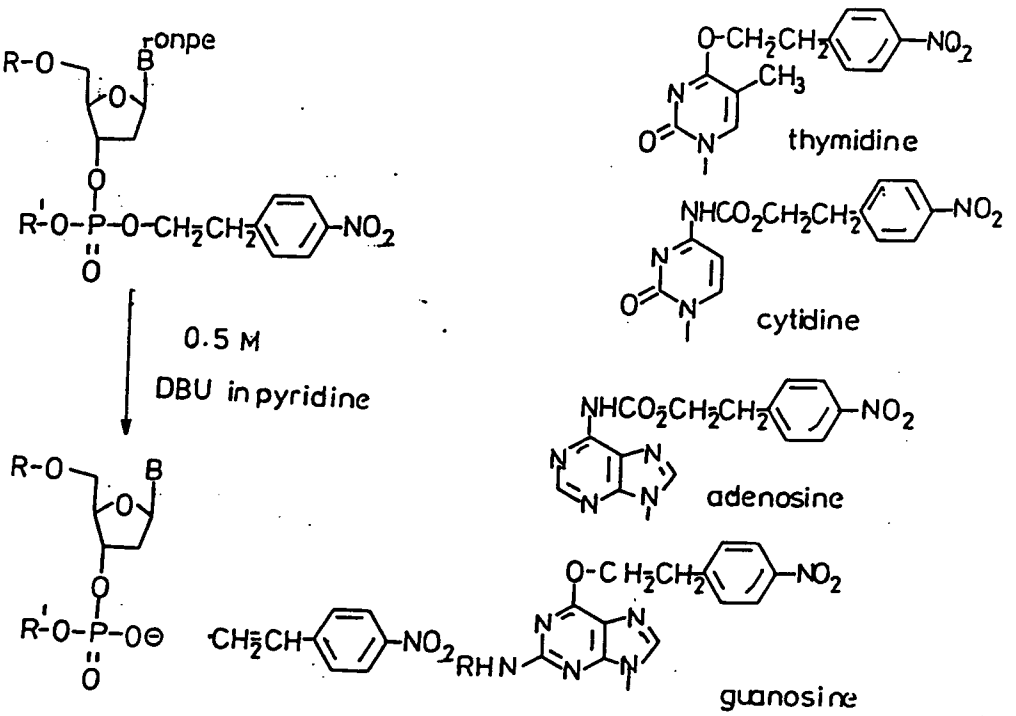
Removal of either (i) dimethoxytrityl group with $ZnBr_2$ or (ii) β-cyanoethyl group with triethylamine will leave either the phosphate (i) or the 5'-hydroxyl (ii) masked so that selective coupling can be achieved between the deprotected sites on the required nucleotides. Deprotection of the o/p-chlorophenyl group is by direct displacement with tetrabutylammonium hydroxide or fluoride ion. The R group can be changed for any of the groups discussed in Section (1.2) including trichloroethyl[100], methylthio[101], 4-nitrophenylethyl[26], 2-phenylsulphonylethyl[102] as well as the 9-fluorenylmethyl group[103]. The range of groups used reflects the desire to achieve mild removal conditions yet involve stable protected intermediates. The cyanoethyl group is known to cause complications due to its lability[104]. Deprotection of the base stable o/p-chlorophenyl group can lead to unwanted side-products due to internucleotide cleavage, as shown in Fig.1.30.

Fig. 1.30



The p-nitrophenylethyl group introduced by Pfleiderer has been used extensively for protection of the internucleotide phosphate and base residues. The general stability to reaction conditions, mild acid or base and its cleavage with a non-nucleophilic base, DBN (known not to affect P-O bond at all) recommend this group for nucleotide synthesis. The adaptability of this group to protect phosphate, and functionalities on the base allows deprotection to be carried out in one step, shown in Fig.1.31.

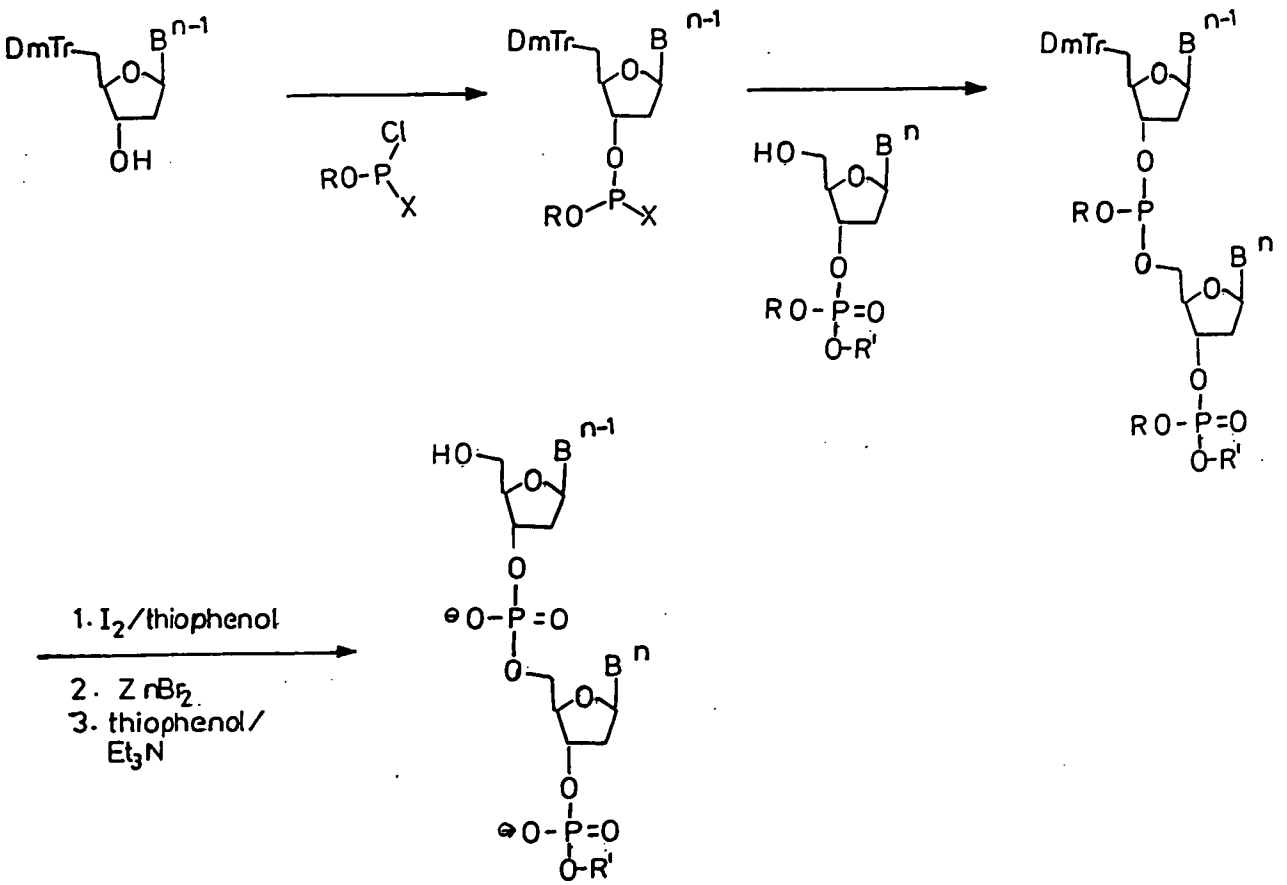
Fig.1.31



However recent work has shown that deprotection is incomplete in 0.5M DBU/pyridine at 50°C (see also Section 1.6) thus complicating purification[105].

The second method of nucleotide synthesis makes use of the extreme reactivity of phosphite intermediates[106], see Scheme

Scheme 2



A methoxy group was employed for phosphite protection when this method was adapted for solid phase synthesis (R=Me, R' =insoluble support). This group, removed with thiophenol/triethylamine, has in general been replaced by the β-cyanoethyl group in automated synthesis.

Many other β-eliminating groups have been examined for phosphite protection including the trichloroethyl[100b], p-nitrophenylethyl, methylsulphonylethyl[101b], 1,1-dimethyl, 2-cyanoethyl[107], benzylsulphonylethyl[108], as well as the 9-fluorenylmethyl[110] group. Some of these can be discounted due to removal conditions requiring strong alkali known to cause internucleotide cleavage. The trihaloethyl groups,

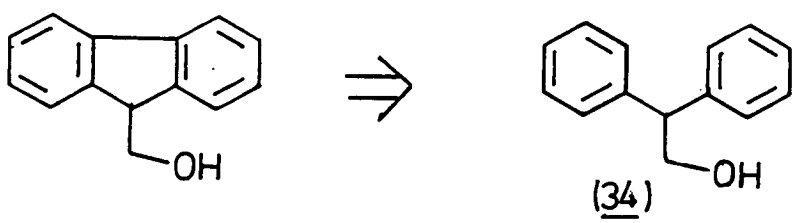
removed with zinc metal or radical anions can present problems due to incomplete removal when used on a solid support[109], whilst the 9-fluorenylmethyl group has reduced coupling efficiency due to steric interference[110].

Interestingly, Claesen, Tesser and Segers[110] have observed uncharacteristic stability of these groups on chromatography with up to 10% triethylamine. They suggest this enhanced stability is due to the phosphite moiety, because of its high electronegativity, being a poorer leaving group than phosphate in an ElcB mechanism endowing the phosphite with increased stability towards bases. The same authors conclude in a study of β-functionalised ethyl groups that the steric hindrance in coupling is of priority, recommending ar(alk)yl sulphonyl groups. However, as will be discussed later (Section 2.8), non-bulky β-eliminating groups are inherently unstable for phosphite protection creating storage problems, unless the ability of the nucleophilic phosphorus to abstract a proton is lowered by bulky substituents[111].

1.6 THE DESIGN OF A NOVEL β-ELIMINATING PROTECTING GROUP

In summary, the topics discussed in this chapter bring together two β-eliminating groups presently widely used in their respective fields. The first was the 9-fluorenylmethoxycarbonyl group which has been used extensively in solid phase peptide synthesis. This group has been very successfully applied and has excellent qualities as a protecting group. However, there have been some doubts raised concerning the lability of Fmoc in the presence of relatively mild organic reagents. Thus, if this group were to be used as the basic structure to begin the design of a novel protecting group then the acidity of the C-H bond would have to be decreased. This can be achieved by simply removing the intermediate bonds tying the phenyl rings together (Section 1.3.iii)

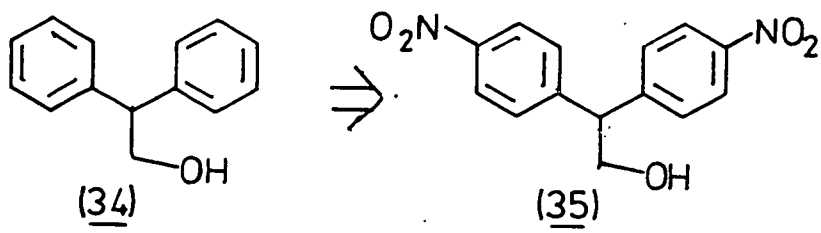
Fig.1.32



(34)

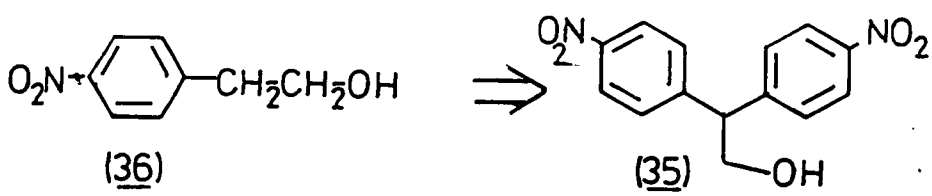
This would produce diphenylethanol (*34*) which has been found to be a very poor β-eliminating[112] group so that the acidity would then require to be increased. This can be achieved by substitution on the phenyl rings with electron withdrawing groups (Section 1.3(iv)) such as nitro groups, as shown in Fig.1.33.

Fig.1.33



This group would be expected to be of about the correct acidity for removal. The same protecting group (*35*) arises from consideration of the p-nitrophenylethyl group which is widely used in nucleotide chemistry. However Ogilvie and Pon have found difficulty on removal of this group even when using 1 molar DBU in pyridine at 50°C for 72 hours[105]. If this group were to be used as a basis for design of a novel protecting group then the acidity requires to be increased. One way of achieving this would be to substitute a β-hydrogen for another p-nitrophenyl group, again producing compound (*35*). We were unaware of the use of (*36*) by Pfleiderer when the initial design studies, based on modification of Fmoc, were carried out. Fig.1.34 illustrates modification of (*36*).

Fig.1.34



Finally, it is worthwhile to note the groups most widely used in physical organic chemistry for mechanistic research have been the 9-fluorenyl derivatives and the 2,2-diarylethylhalogen series (see Sect.1.3).Of the latter series

the substituent combination of most topical interest has been the 2,2-bis(4-nitrophenyl)ethyl moiety. There is some controversy whether elimination of this group is *via* an ElcB-limiting or ElcB-like E2 mechanism. 9-Fluorenylmethyl chloride is known to undergo an E2 type elimination. Assuming the leaving group effect is similar then the implication is that the bis(4-nitrophenyl)ethyl group should have acidity similar to, or slightly lower than, that of the fluorenyl derivatives.

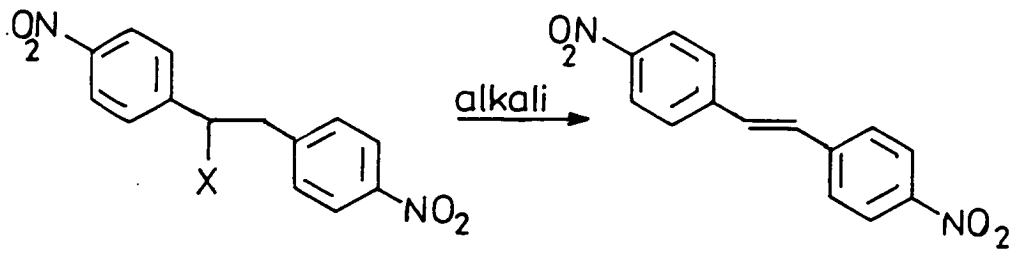
For the successful introduction of a novel protecting group stringent criteria have to be achieved. The removal of the group must be selective and yet achieved under mild conditions. It is the balance between these two factors which play the most important role in defining the final usefulness of a prospective protecting group. However for complete success all of the following conditions must be met.

(1) High cleavage yield under mild conditions.
(2) Ease of introduction.
(3) Compatability with other protecting groups.
(4) Should neither possess nor introduce a chiral centre.
(5) Reagent(s) for introducing it should be readily available and non-toxic.
(6) It should be readily separable from the deprotected compound.
(7) The cost must be considerably less than that for Fmoc derivatives and, if possible, approach the cost of Boc derivatives.

1.6.1 2,2-bis(4-Nitrophenyl)ethanol

A survey of the literature produced no references to this compound. However similar compounds do exist such as the isomer 1,2-bis(4-nitrophenyl)ethanol.

Fig.1.35



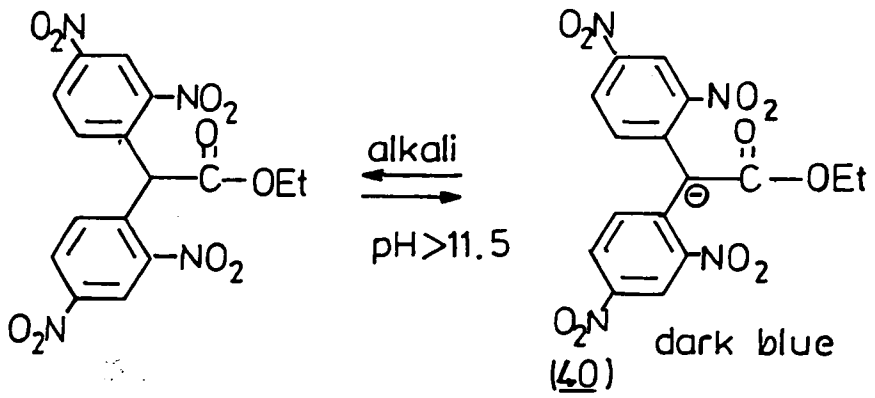
(37) (38)

This compound has been studied for the physical properties giving rise to intramolecular hydrogen bonding between the hydroxyl proton and the 2-aryl group[113]. A study into the mechanism of elimination to give trans-4,4-dinitrostilbene in aqueous dioxan and alkali is thought to be E2[114].

The olefinic compound 1,1-bis(4-nitrophenyl)ethene (*39*) is a very well known and studied material. It was first synthesised in 1948 by Lorenz[115] and has been used as a radical quencher[116]. The structure of (*39*) has been determined by X-ray crystallographic analysis in 1974[117].

The methyl and ethyl esters of 2,2-bis(2,4-dinitrophenyl) acetic acid have a very long history being first synthesised in 1888[118]. Their use was recognised in 1946 by Canbäch[119] who used their ability to form a dark blue colour in alkali as an indicator.

Fig.1.36



(40)

The 2,2-bis(4-nitrophenyl)ethyl halides discussed in Section (1.3) have been used as a tool to study elimination mechanisms. As well as the di and trihalide derivatives discussed in Section (1.3) the monohalide derivatives, 2,2-bis(4-nitrophenyl)ethylfluoride[67] and chloride[115] are known. The former used in the same study as the trifluoro derivative into elimination mechanism. However the alcohol (*35*) and derivatives thereof are unknown.

CHAPTER 2

DISCUSSION

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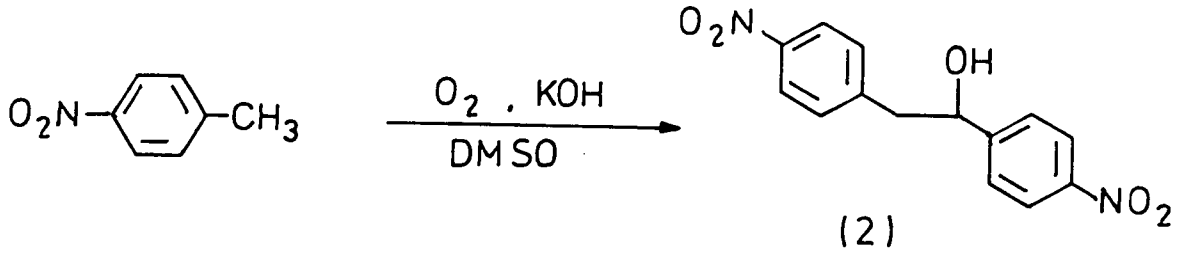
2.1 SYNTHESIS OF 2,2-BIS(4-NITROPHENYL)ETHANOL (*1*)

The synthesis of 2,2-bis(4-nitrophenyl)ethanol (*1*) was initially investigated by comparison with the synthesis of the structurally similar compounds discussed in Section 1.6. By adaption of one of these routes it was hoped that a method leading to (*1*) could be derived. The first compound to be considered was the isomer 1,2-bis(4-nitrophenyl)-ethanol (*2*). This compound was obtained starting from p-nitrotoluene.

Scheme 2.1

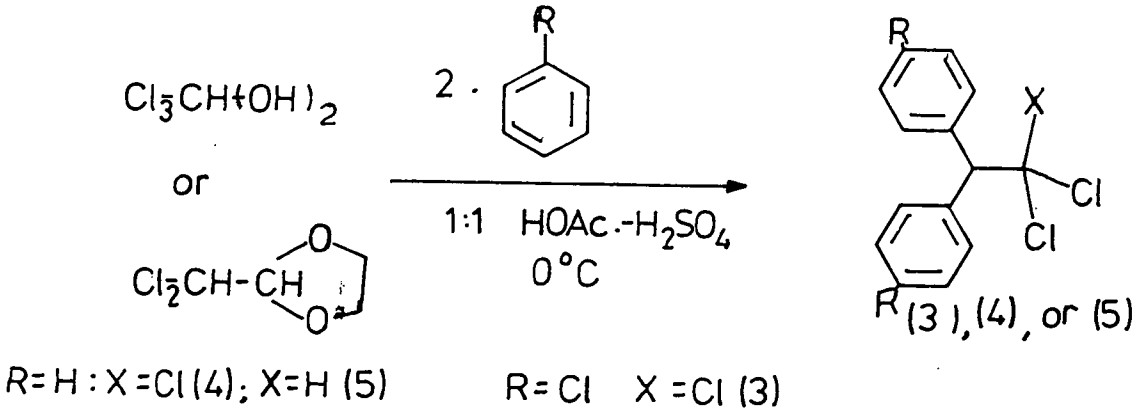


(2)

The reaction is thought to proceed by oxidation of p-nitrotoluene to p-nitrobenzaldehyde followed by attack of the p-nitrobenzyl anion to produce the alcohol (*2*)[120].

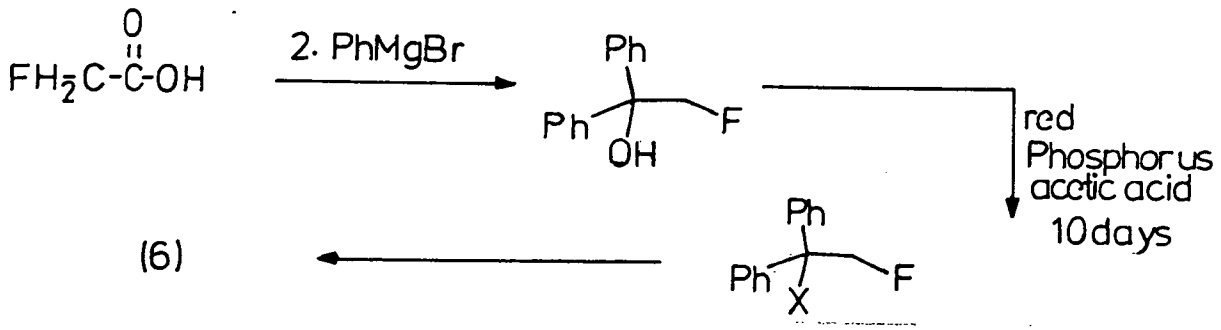
The halide derivatives discussed in Section 1.3.2 were the second type of related compound illustrated in Section 1.6. These derivatives have been obtained through modifications of the 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (*3*) (DDT) synthesis[121] shown below (Scheme 2.2).

Scheme 2.2



Nitration of either (*4*) or (*5*) produced the respective p-nitro derivatives. Fry and Pulay have used a slightly modified route to obtain 2,2-bis(4-nitrophenyl)-1,1-dichloroethane using acetyl chloride in a Friedel-Crafts acylation with benzene to give acetophenone after which the method was similar to Scheme 2.2[65]. 2,2-Bis(4-nitrophenyl)-ethylfluoride (*6*) was also obtained starting from an acetic acid analogue[67], Scheme 2.3 where X=H, or D if deuterated acetic acid was used in reduction.

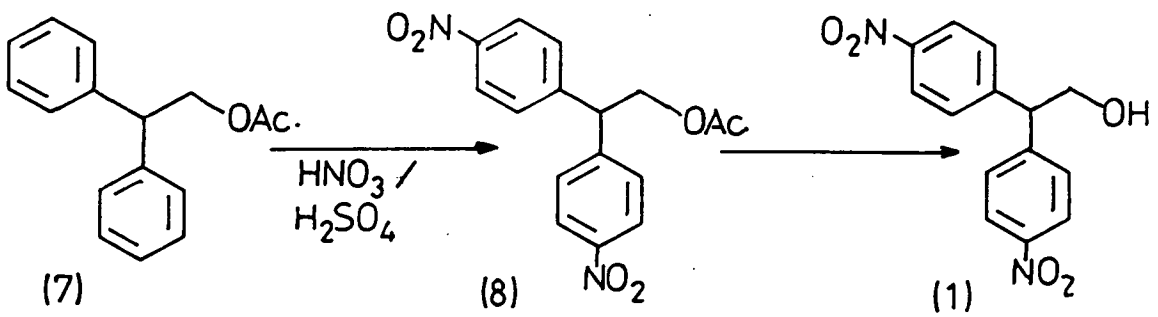
Scheme 2.3



These routes (Schemes 2.2 and 2.3) have provided flexible syntheses to labelled material for use in mechanistic research. However they do not provide a practical method for large scale synthesis of the alcohol (*1*) although they do demonstrate the ability to nitrate 2,2-diphenylethyl derivatives to produce the 4,4'-

substitution pattern. It would appear logical that a similar nitration of the acetate (*7*) of 2,2-diphenylethanol should yield 2,2-bis(4-nitrophenyl)ethanol (*1*) after hydrolysis of (*8*) (see Fig.2.1).

Fig.2.1



(7) (8) (1)

We decided to investigate the nitration of (*7*) by three nitration methods, i.e. with mixed acids in nitromethane, acetyl nitrate and direct addition to mixed acids.

The first method, used by Tashiro and co-workers[122] for the nitration of diphenylmethane produced the nitrated acetate (*8*) in pure form but in poor yield (∿30%). The remaining material consisted mainly of the <u>para</u> substituted derivative along with two other compounds possessing almost identical (H.P.L.C.) retention times. Purification of this material proved impossible.

The second method relied upon the mild nature of acetylnitrate as a nitrating species[123]. Acetyl nitrate, obtained by the addition of concentrated nitric acid to acetic anhydride at 0°C, was added to 2,2-diphenylethyl acetate (*7*) in acetic anhydride at -10°C. The reaction was warmed to room temperature over 30 minutes and on work-up produced the acetate (*8*) in 50% yield. Varying the reactant concentrations and the reaction temperature failed

to improve upon this yield although by t.l.c. no other products were observed. Analysis of the acetate (*8*) produced by this method and of the subsequently produced alcohol (*1*) by i.r., t.l.c., H.P.L.C. proton n.m.r. and melting point indicated pure material had been obtained. However, later analysis of the ^{13}C spectra showed a double peak to be present for the methylene proton at 64.5 ppm. The use of this material in later syntheses did not affect the course of the reaction or the yields, however after work-up and isolation of the product it was found impossible to crystallise compounds made using this material. The instability of acetic anhydride and nitric acid solutions also provided reason to discontinue this route.

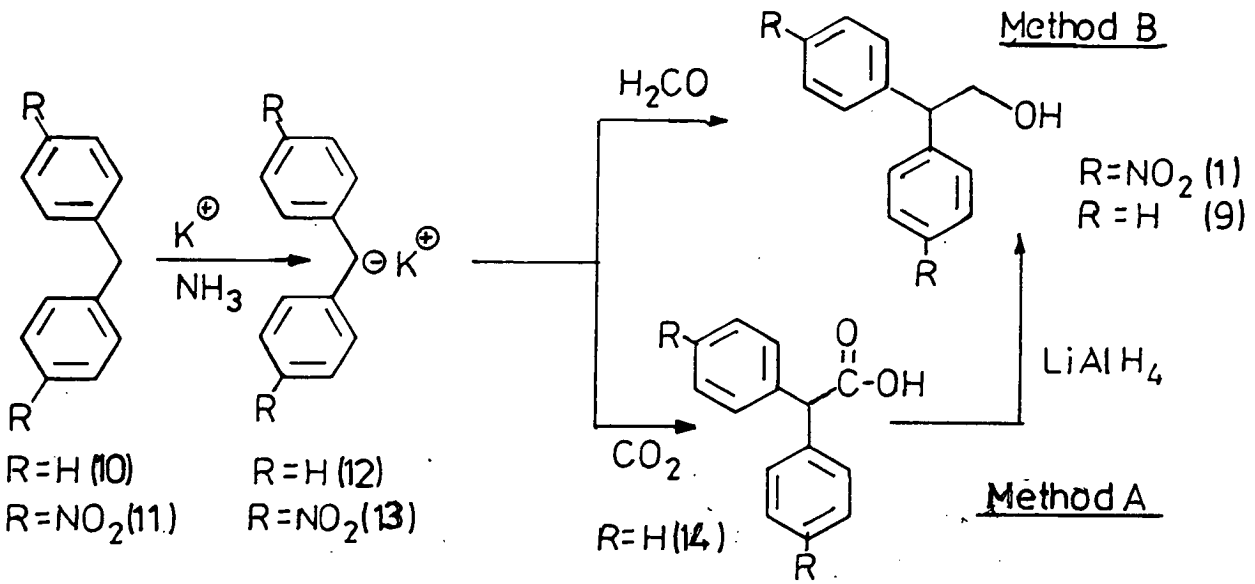
The third nitration method investigated was by addition of solid 2,2-diphenylethyl acetate (*7*) to a vigorously stirred mixture of concentrated nitric and sulphuric acid at -10°C.[121] This route produced a gum which, after neutralisation with water and dilute caustic solution, gave crystalline nitrated acetate (*8*) in a yield of 75%. Recrystallisation of this material from chloroform followed by hydrolysis in refluxing methanolic hydrochloric acid produced pure 2,2-bis(4-nitrophenyl)ethanol (*1*) in good overall yield of 60% (based on 2,2-diphenylethyl acetate). This is currently the method of choice and has given consistently pure alcohol (*1*).

2.1.2 Synthesis of 2,2-diphenylethyl acetate (*7*)

The method described above provides a useful route from the acetate (*7*) to the alcohol (*1*). It was therefore desirable to obtain a synthesis which provided (*7*) in large

amounts from cheap starting materials and reagents. A survey of the literature suggested two general strategies to this compound outlined in Schemes 2.4 and 2.5.

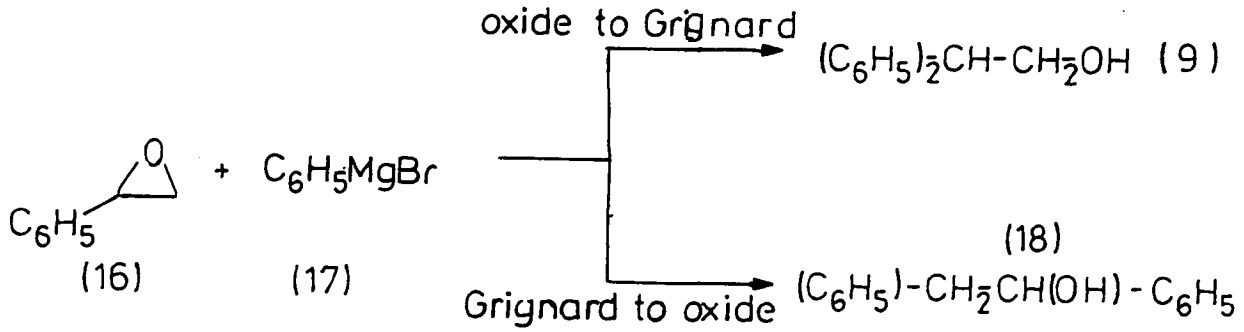
Scheme 2.4



Scheme 2.4 sets out the approach of Hamrich and Hauser[112] and provides 2,2-diphenylethanol (*9*) either directly by formylation of potassium diphenylmethide (*12*) or by reduction of 2,2-diphenylacetic acid (*14*) which is produced on quenching (*12*) with carbon dioxide. These authors claim the former to be the preferred route. We repeated this work using potassamide[124] in ether to which was added diphenylmethane (*10*) in ether. Formylation was attempted by cracking paraformaldehyde (mixed with phosphorus pentoxide to ensure anhydrous condition) by heating and passing the gaseous formaldehyde, driven by a nitrogen stream, into the stirred solution of (*12*) for up to thirty minutes. However no product was isolated from this route or by using sodamide instead of potassamide. In all cases diphenylmethane (*10*) was reisolated in 90% yield. The substrate

was changed to di(4-nitrophenyl)methane (*11*) in an attempt to increase the ease of formation of the carbanion intermediate (*13*)[48]. However very little material was obtained by this route. The second route (Method A, in Scheme 2.4) produced diphenyl acetic acid (*14*) in very good yield (90-95%) on quenching the anion (*12*) with carbon dioxide. The alcohol (*9*) was obtained after reduction of the methyl ester (*15*) of (*14*) with lithium aluminium hydride giving a 84% yield. 2,2-Diphenylethyl acetate (*7*) was produced by stirring (*9*) in pyridine with a slight excess of acetic anhydride for 4 hours. Nitration of (*9*) as described in Section 2.1 followed by hydrolysis yielded 2,2-bis(4-nitrophenyl)ethanol (*1*). This scheme (Method A) provides good yields of pure alcohol (*9*) in quantities required for the initial development of the alcohol (*1*) and has the advantage that all purification is achieved by crystallisation and no ambiguity exists in the product obtained (c.f. Scheme 2.5).

Scheme 2.5

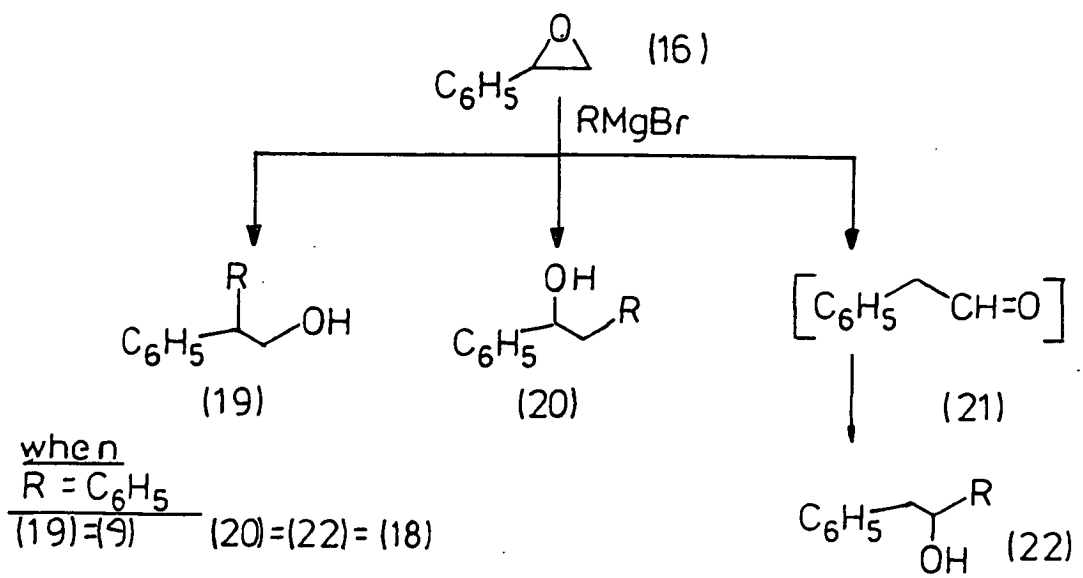


The second approach we have investigated for the synthesis of alcohol (*9*) is shown above (Scheme 2.5). Kharasch and Clapp[125] have described the opening of styrene oxide with phenylmagnesium bromide to give either the expected secondary alcohol (*18*) after attack at the least hindered

centre or the primary alcohol, (*9*), depending on the order of addition of the Grignard reagent. The primary alcohol (*9*) can be obtained in about 80% yield, together with 20% of the other isomer, if the oxide (*16*) is added to the Grignard. A reversal of these ratios is obtained if the Grignard is added to the oxide. The former route has been repeated by Wigham[161a] to give yields of around 70% crystalline material (*9*)with a further 12% isolable from the supernatant after silica chromatography.

The course of the reaction leading to the 2,2-isomer rather than the 1,2 adduct requires some attention.

Scheme 2,6

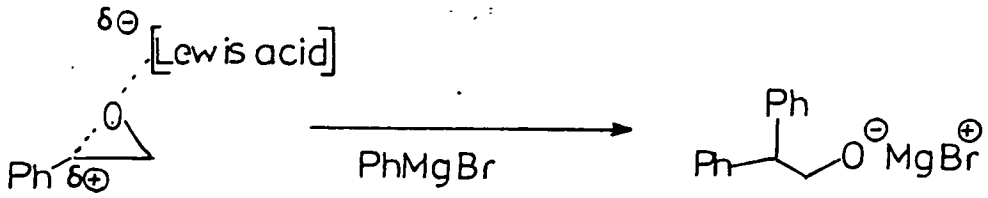


Nucleophilic attack on (*16*) can occur at either the more accessible site to form (*18*) or at the more electrophilic carbon to form (*9*). Nucleophilic reagents with sufficient Lewis acidity can cause rearrangement of styrene oxide to the aldehyde intermediate (*21*) followed by nucleophilic attack to give the secondary alcohol (*22*). The yield of (*22*) is greatest when reagents such as $R_2Cu(CN)Li_2$ [126] are

used. Grignard reagents such as methylmagnesium bromide can cause significant amount of isomerisation of (*16*) to give (*22*). The highest yield of isomer (*19*) has been reported with dialkylmagnesium (*23*) forming (*19*) to the exclusion of the other isomer.[127] Dialkylmagnesium is formed by disproportionation of the Grignard reagent and has been reported to be present in all Grignard reactions to some extent. The equilibrium can be affected by temperature with the amount of (*23*) increasing from about 60% to 70% on raising the temperature from -15°C to 35°C[128]. Reagent (*23*) can be obtained quantitatively from the Grignard mixture at reflux, by precipitating out the halide containing material with dioxan leaving (*23*) in solution[128,129]. However when this method was attempted with phenylmagnesium bromide (*24*) and styreneoxide a mixture of compounds was obtained which contained none of the desired alcohol (*9*). The identity of the products of this reaction was not pursued since by i.r. no alcohol peak was observed.

The original scheme (2.5) is currently being reinvestigated and should provide more promising results. With monosubstituted epoxide, such as (*16*), nucleophilic attack can occur at either site. In neutral and basic conditions attack at the less sterically hindered site is preferred. However under acid conditions (or in the presence of weak Lewis acid) nucleophilic attack will be preferred at the centre with most carbocation character, (see Fig.2.4).

Fig.2.4

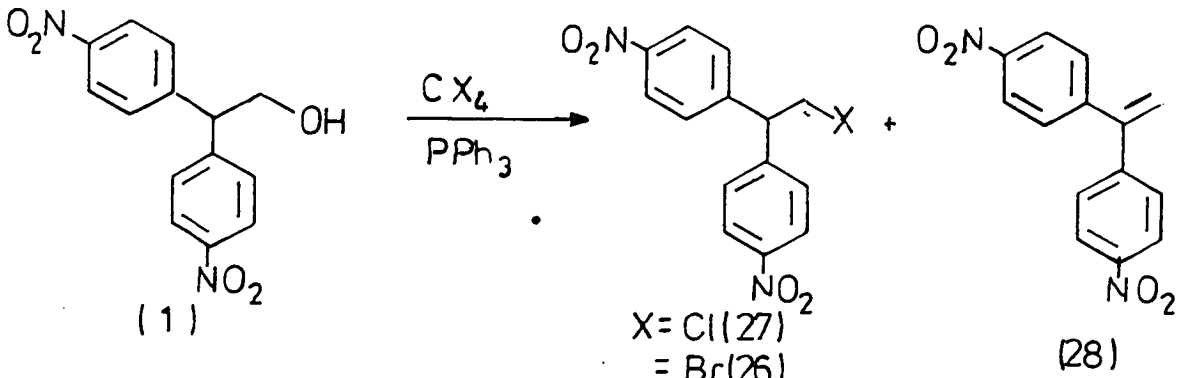


2.2 SYNTHESIS OF DERIVATIVES OF 2,2-BIS(4-NITROPHENYL)-ETHANOL (*1*)

2.2.1 2,2-Bis(4-nitrophenyl)ethyl halides

The synthesis of 2,2-bis(4-nitrophenyl)ethyl bromide (*26*) was first attempted using alcohol (*1*) stirred with phosphorus tribromide (*25*) in dichloromethane at 0°C for 2.5 hours at room temperature. This method afforded no bromide (*26*), with the alcohol being reisolated. A second approach proved more successful with reaction of the alcohol (*1*) with carbon tetrabromide and triphenylphosphine in dichloromethane to give bromide (*26*) in 70% yield as a crystalline solid after silica chromatography. The 2,2-bis(4-nitrophenyl)ethyl chloride (*27*) was synthesised analogously using carbon tetrachloride as both solvent and reagent, however purification was complicated by the production of 1,1-bis(4-nitrophenyl)ethene (*28*) which possessed the same Rf on silica chromatography thereby reducing the yield of (*27*) to 50% after crystallisation. The halides were required for future prospective work on protection of β-lactam and nucleoside functionalities.

Fig.2.5

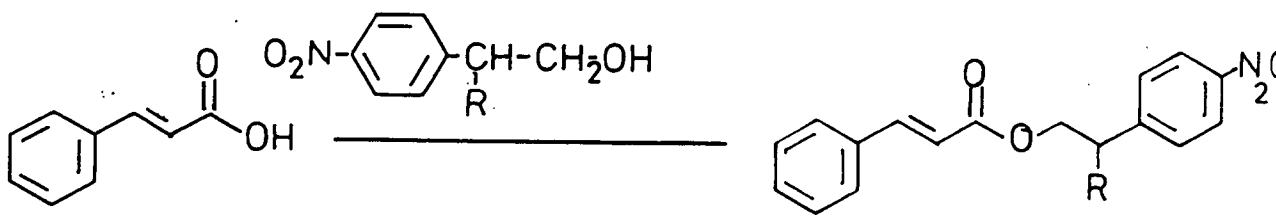


The olefin (*28*) could be separated from the reactions and identified using standard techniques such as silica chromatography. Alternatively it could be prepared directly by the elimination of acetic acid from acetate (*8*) with diazabicyclo[4.3.0]non-5-ene (DBN), see Section 2.4, to provide the pure olefin (*28*) in quantitative yield after routine base and acid washing.

2.2.2 Ester formation

It was found that 2,2-bis(4-nitrophenyl)ethanol (*1*) can form esters without any difficulty or observed olefin (*28*) formation. This is demonstrated by the synthesis of 2,2-bis(4-nitrophenyl)ethyl cinnamate (*29*) with dicyclohexylcarbodiimide and a catalytic amount of 4-dimethylaminopyridine (DMAP). The cinnamate (*29*) was obtained crystalline and in good yield (70%).

Fig.2.6



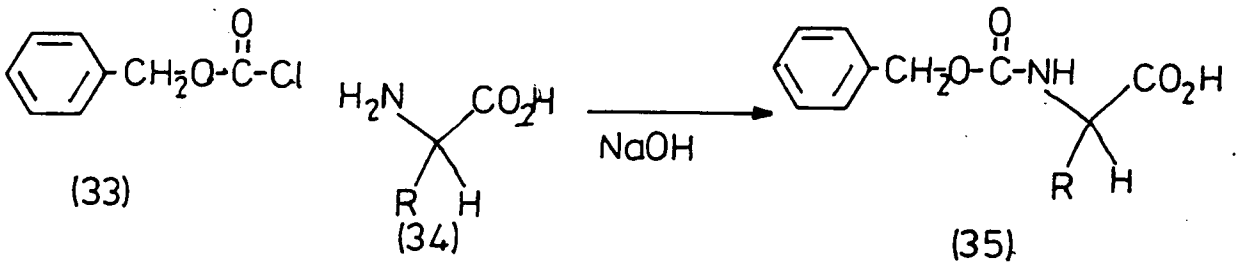
R=H (32), R=O$_2$N-C$_6$H$_4$ (1) / R=H (31), R=O$_2$N-C$_6$H$_4$ (29)

The same conditions were used for the synthesis of crystalline 2-(4-nitrophenyl)ethyl cinnamate (*31*) obtained in good yield (85%) from 2-(4-nitrophenyl)ethanol (*32*) and cinnamic acid (*30*). These compounds were later used as models for comparative stability studies.

2.2.3 Synthesis of 2,2-bis(4-nitrophenyl)ethyl chloroformate (*37*)

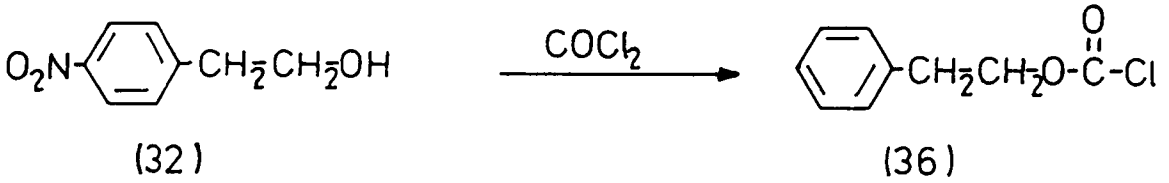
Nα protection of aminoacids has been achieved predominantly using urethane derivatives prepared via chloroformates, see Fig.2.7, in turn, derived from the alcohol. Reaction of the chloroformate with the amine is conveniently carried out under Schotten-Baumann conditions.

Fig.2.7



We set about the synthesis of the chloroformate derivative of alcohol (*1*) by following standard reaction conditions as described by Pfleiderer for his synthesis of (*36*),[26] shown in Fig.2.8.

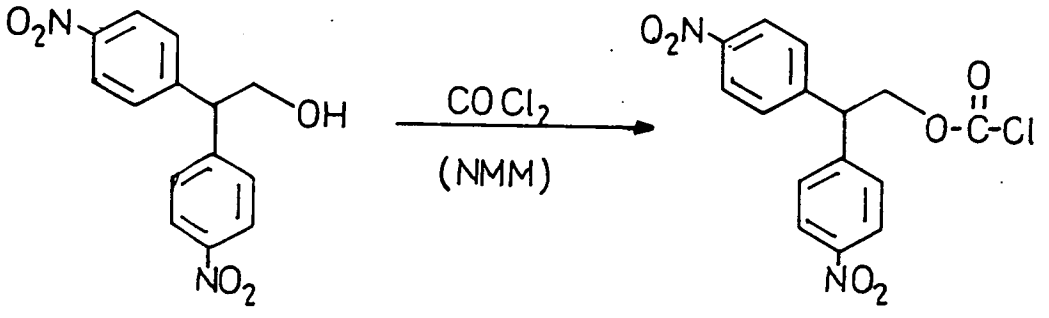
Fig.2.8



The alcohol (*1*) was added to a stirred, chilled solution of excess phosgene in toluene over thirty minutes and stirring was continued for one hour, after which the temperature was raised to 50°C for 5 hours. Using these conditions Pfleiderer obtained (*36*) in quantitative yield,

however using alcohol (*1*) no reaction was observed by infra-red. Repeating this experiment using a greater excess of phosgene had little effect, as did raising the temperature under an argon or nitrogen atmosphere. It is required that the reaction should go to completion as separating a mixture of starting material and product would be difficult due to the sensitivity of the chloroformate (*37*) on silica.

Fig.2.9

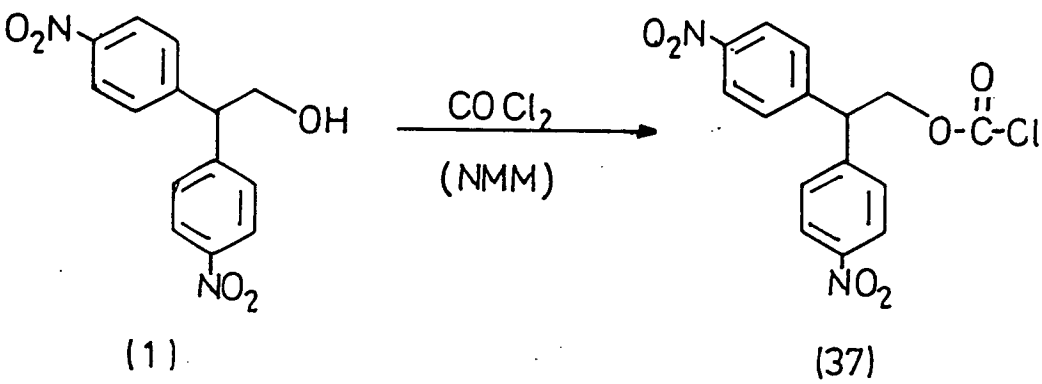


(1) (37)

A second approach was to add a tertiary amine to increase the reactivity of the alcohol (*1*) by proton abstraction and remove the hydrochloric acid produced in the reaction. Addition of N,N-dimethylaniline significantly increased the yield of (*37*) as indicated by a large peak at 1780 cm^{-1} in the infra-red spectrum. However it was not possible to form the chloroformate (*37*) quantitatively from the alcohol (*1*) even after further addition of base and phosgene. Repeating the reaction using N-methylmorpholine in place of N,N-dimethylaniline brought about complete reaction to give the chloroformate (*37*) within five minutes of base addition to the alcohol (*1*), indicated by the

formation of a large peak at 1780 cm^{-1} and the disappearance of the alcohol peak at 3600 cm^{-1} in the infrared spectrum. The hydrochloride salt was filtered off and the solvent removed in vacuo to produce 2,2-bis(4-nitrophenyl)ethyl chloroformate (*37*) in quantitative yield as a white crystalline solid, see Fig.2.9.

Fig.2.9

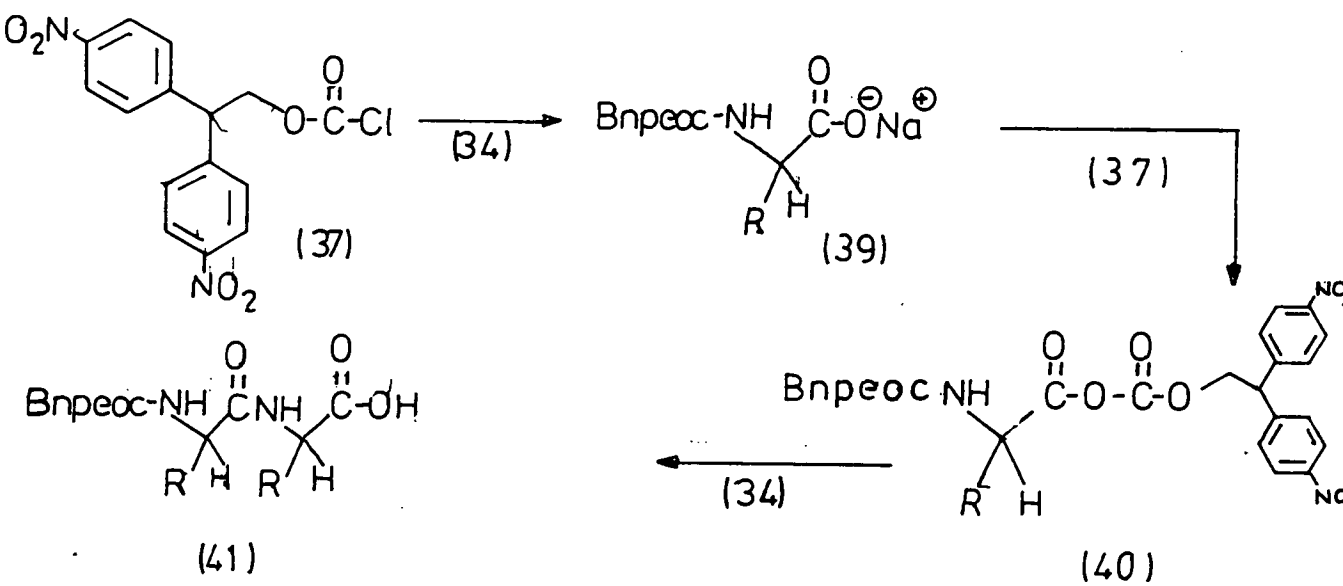


(1) (37)

2.2.4 Protection of amino acids

The chloroformate (*37*) was then used to protect the amino function of alanine (*38*) by the Schotten-Bauman method. Under these conditions (*37*) in dioxan was added to (*38*) dissolved in an equivalent of sodium hydroxide. The pH was kept constant at pH 10.5 by addition of alkali. The reaction was stopped by adjusting the pH to 1.5 and extracting the products into ethyl acetate. A t.l.c. of the crude reaction mixture indicated a number of products the most significant contaminant of the desired material (*39*), was found to be the dipeptide (*41*). The formation of which can be rationalised via a mixed anhydride intermediate (*40*) (see Fig.2.10).

Fig.2.10

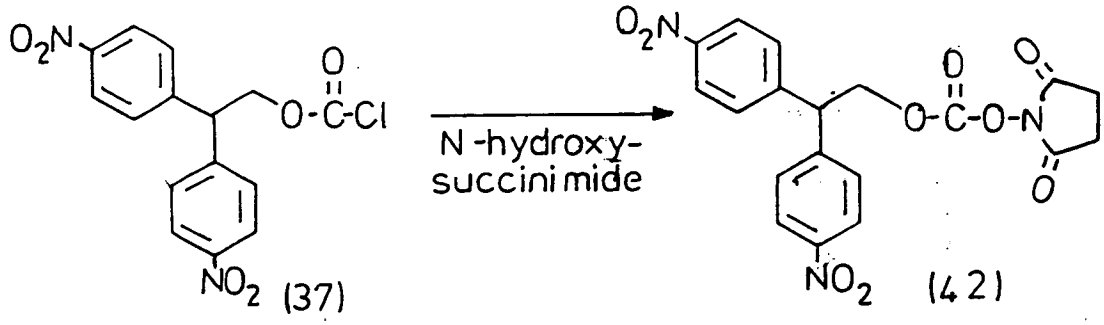


The formation of (*41*) by this route is influenced by pH, temperature and the relative concentrations of the reactants. Changing these parameters would probably reduce the amount of dipeptide formed[130]. However, oligopeptide formation during protection of an aminoacid has been observed by many workers, especially with non-functionalised aminoacids, such as glycine or alanine. Protection using Fmoc-chloride has been shown to give up to 20% oligopeptide formation with a wide range of aminoacids.[131] Similar findings have been obtained using benzyloxycarbonylchloride (ZCl) for introduction of the Z-group; and for the tert-butyl-oxycarbonyl group when using di-tert-butyl-dicarbonate[132,133].

This problem has led to the development of other ac ylating reagents which would react selectively with the primary amine. Following the use of N-succinimidyl carbonate

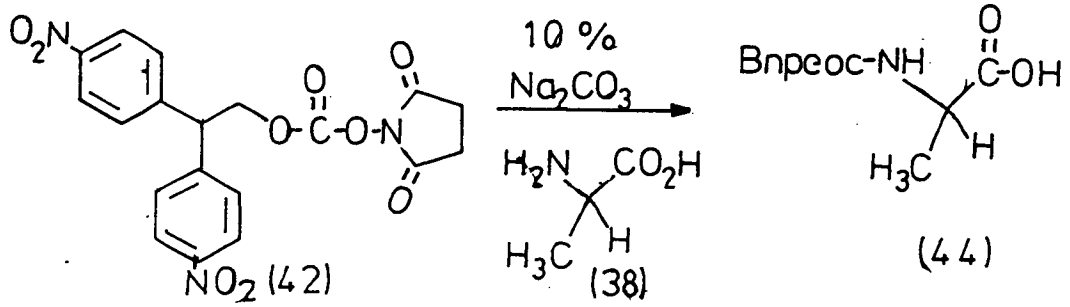
by Paquet in 1982[134] for amino protection, a large number of derivatives based upon mixed carbonates have been investigated.[135-139] Of the groups used to date the N-succinimidyl carbonate appears to offer the best characteristics, combining high acylation yields with ease of preparation and use. Lapatsanis and co-workers have used this group to protect amino acids bearing free side-chain hydroxyl groups with Fmoc[138]. These desirable features led us to investigate the possibility of the Bnpeoc N-succinimidyl carbonate (*42*) overcoming the problems encountered earlier with dipeptide formation. This should also provide a reagent capable of protecting different types of amino acids including hydroxyl-containing residues (i.e. serine, threonine and tyrosine). The succinimidyl derivative (*42*) (see Fig.2.11) was prepared by the dropwise addition of NMM to a solution of the chloroformate (*37*), and N-hydroxysuccinimide (*43*) in dioxan at 0°C. Stirring was continued for thirty minutes after which the amine hydrochloride salt was filtered off. Removal of the solvent afforded pure N-succinimidyl carbonate (*42*) as a white solid from ethyl acetate (Method A). Adaptation of this route using dichloromethane as the solvent for the preparation of the chloroformate (*37*) allowed the synthesis to proceed without isolation of (*37*), this produced (*42*) in overall yield of 75% based on the alcohol (*1*) (Method B).

Fig.2.11



Protected alanine was prepared by the addition of Bnpeoc ONSu (*42*) in dimethylformamide to a slight excess of free alanine (*38*) in 2 equivalents of a 10% sodium carbonate solution at 0°C (see Fig.2.12). Work-up of the reaction and removal of the solvent provided pure N-[2,2-bis(4-nitrophenyl)ethoxycarbonyl]-L-alanine (*44*) which crystallised from chloroform/diethyl ether in 89% yield.

Fig.2.12



Protection using N-succinimidyl carbonate (*42*) has been generally applied with other amino acids and with-standing a few notable exceptions (to be discussed) has provided high yields of pure and crystalline derivatives. A complete summary of Bnpeoc amino acids together with melting points and specific rotation is given in Table 2.1.

A slightly modified route was used for amino acids bearing

acid-labile side-chain protection or acid-sensitive amino acids in which case saturated citric acid was used instead of conc. hydrochloric acid during work-up.

Protection of 7-aminocephalosporanic acid and 6-aminopenicillanic acid with Bnpeoc ONSu (*42*) resulted in very poor yields of the respective Bnpeoc acids (*59*) and (*60*), this was thought to be due to the reduced nucleophilicity of these amines. Preparation of Bnpeoc aminoisobutyric acid (*61*) was also associated with very poor yields when using reagent (*42*) due to the sterically hindered nature of this amino acid. For these types of amino acids it is necessary to use the chloroformate (*37*) to achieve protection. The greater reactivity of (*37*) over (*42*) compensates for the problems associated with sterically hindered amino acids providing protection in much improved yields.

The protection of methionine and tryptophan required the reaction to be carried out with exclusion of oxygen. Both reaction and work-up were performed under nitrogen to provide respectively Bnpeoc protected amino acids (*62*) and (*63*) in good yield. Characterisation of these and other selected amino acids could be achieved as their respective dicyclohexylamine salts (*64*) and (*65*). The use of a secondary amine to form salts was not associated with any difficulties such as elimination. The free acid could be regenerated by chromatography on silica.

Some difficulty was encountered with the protection of tyrosine (*66*) with Bnpeoc ONSu (*42*) in sodium carbonate solution. This route afforded Bnpeoc tyrosine (*67*) in disappointing yields (<30%).

A second approach used an extra 5% sodium bicarbonate as a buffer to counter the acidic nature of the phenolic function. This led to an increase in yield of (*67*) to 40% but this was still low in comparison with other amino acids. Protection with Bnpeoc-Cl (*37*) was not attempted as acylation of the phenolic hydroxyl group would occur. Another difficulty was associated with the poor solubility of (*66*) in sodium carbonate solution. However this problem was overcome when the base was changed from sodium carbonate to sodium hydroxide. This synthesis provided (*67*) in good yield and pure after silica chromatography. The slight impurity consistently produced by this method is thought to be the O-acylated tyrosine, as the phenolic hydroxyl group is known to be a good nucleophile in basic conditions[140].

Fig.2.13

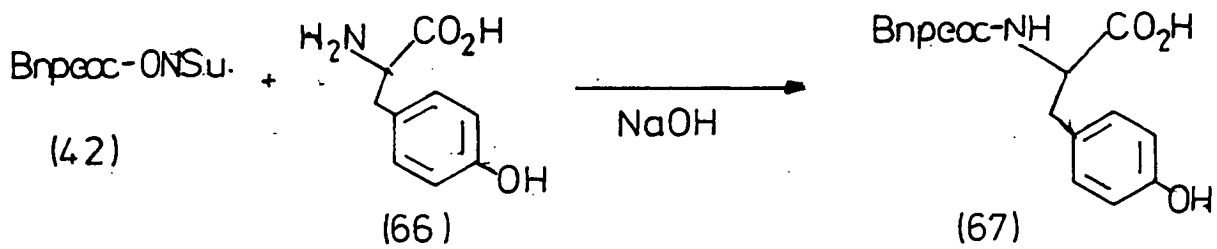


Table 2.1 provides a summary of the melting points and specific rotation (in dimethylformamide with C=1)[161a] of the Bnpeoc amino acids discussed in this section.

Table 2.1

	m.p.°C;	$[\alpha]_D^{25}$ (C=1, DMF)°			m.p.°C;	$[\alpha]_D^{25}$ C=1, DMF)°
Aib	166-169	-		Leu	68-71	-4.1
Ala	148-149	+0.7		Lys(Z)[161e]	95(d)	+1.3
Arg(Pmc)[161f]	133-137**	+5.8**		Met	179-181*	+8.1
Asn	177-178	+0.5		Phe	79-80	-15.7
Asp(OBu^t)[161g]	146-147	+2.9		Pro	94-96*	-13.6*
Gln	185-187	-3.9		Ser	169-173	+3.1
Glu(OBu^t)	125-126*	+5.2*		Thr	171-173	+3.8
Gly	156-158	-		Trp	169-170*	-1.5*
His(HCl)[161e]	127(d)	-24.5		Tyr	171-173*	+25.6*
Ile	169-171*	+7.7*		Val	148-150	+6.2
Arg(NO_2)	-	+0.3		APA	-	+11.9
				ACA	-	-46.9

** Cyclohexylamine salt; * Dicyclohexylamine salt

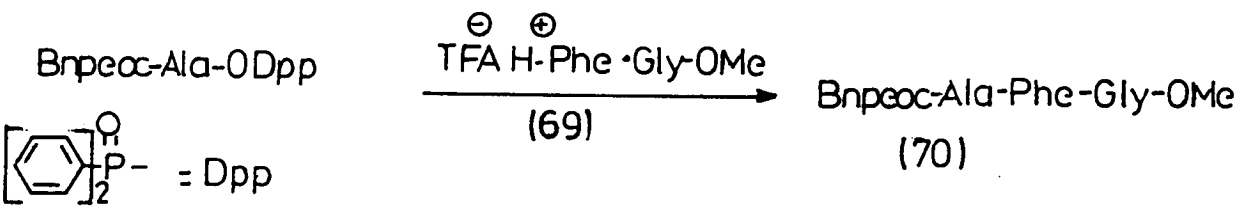
Other methods for forming urethane protected amino acids using N-succinimidyl carbonate derivatives can be useful in circumstances where solubility of the base or the carbonate is a problem. In such cases the use of acetonitrile and triethylamine has been described in place of sodium carbonate in dioxan or dimethylformamide[137,139,141].

2.2.5 Peptide synthesis in solution phase

It was important to evaluate the ability of these novel protected amino acids to form peptide bonds under standard conditions. For this purpose the tripeptide N-[2,2-bis(4-nitrophenyl)ethoxycarbonyl]alanylphenylalanyl glycine methyl ester (*70*) was synthesised. This peptide

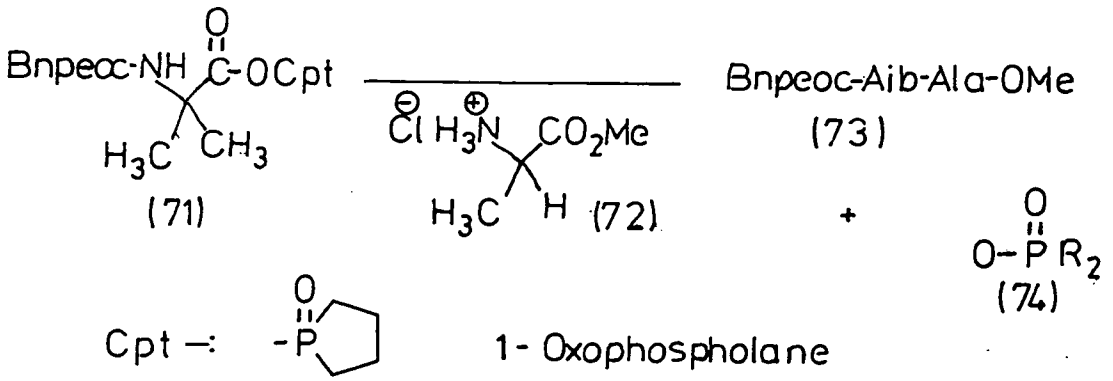
was obtained through coupling of the diphenylphosphinic mixed anhydride of Bnpeoc Ala-OH (*44*) with the TFA salt of H-Phe-Gly-OMe (*69*) in the presence of an equivalent of N-methylmorpholine and 2,6-lutidine in dichloromethone (see Fig.2.14). After stirring for an hour at 0°C and an hour at room temperature, work-up of the reaction followed by chromatography on silica produced pure (*70*) which crystallised from chloroform diethyl ether in 75% yield.

Fig.2.14



The synthesis of a second peptide N-[2,2-bis(4-nitrophenyl)ethoxycarbonyl aminoisobutylalanine methyl ester (*73*) was then attempted to evaluate the ability of activated Bnpeoc amino acids to form sterically hindered peptide bonds. 1-Oxophospholane chloride (Cpt-Cl) was used to form the mixed anhydride thus allowing the course of the reaction to be monitored by ^{31}P-n.m.r. A second objective of this experiment was to judge the thermal stability of the aforementioned mixed anhydride.

Fig.2.15



The reaction was performed in a n.m.r. tube and ^{31}P n.m.r. spectra obtained at appropriate intervals. The mixed anhydride (*71*) was formed at 0°C and found to be stable when warmed to 30°C. Addition of the salt (*72*), NMM and 2,6-lutidine at 30°C was followed by subsequent warming to 50°C at which time a small amount of product (*74*) was observed by the formation of a peak at δ64.38. A ^{31}P spectrum after 14 hours showed the reaction to be about 60% complete, as indicated by the slow disappearance of the peak at δ76.2 (*71*) to be replaced by the peak at δ64.5 due to (*74*). After work-up and purification by preparative t.l.c. the dipeptide (*73*) was identified by proton n.m.r.

2.3 STABILITY STUDIES

2.3.1 Introduction

The use of different reaction conditions and reagents in peptide synthesis requires a prospective protecting group to be stable towards a wide variety of conditions. In the previous section (Section 2.2) the Bnpeoc group was demonstrated to form peptide bonds in normal fashion, implying the stability of this group to commonly used reagents. It must however be susceptible to fast and efficient cleavage. To investigate whether the Bnpe(oc) group possesses these qualities the acetate (*8*), cinnamate (*29*) and tripeptide (*70*) were used as model compounds and their respective stability measured against a wide variety of conditions.

2.3.2 Stability towards base

As has been discussed, β-eliminating groups are designed to be labile towards base. The Fmoc-group is removed with a secondary amine (excess 20% piperidine in dimethylformamide) whilst the p-nitrophenylethyl group is removed with DBN in pyridine. The stability of the Bnpe-group towards these and three other bases (listed in Table 2.2) which are commonly used in organic synthesis was tested by addition of 1 equivalent of the required base to a solution of Bnpe acetate (*8*) in deuterated dichloromethane (0.3M) and the reaction monitored by proton n.m.r. Any olefin (*28*) formation was observed by a characteristic peak at 5.8 ppm, well removed from other peaks. From the spectra the following results were obtained.

Table 2.2

BASE	Elimination of acetate (*8*) in CD_2Cl_2			
	2 min	7 min	3 hr	24 hr
DBN	75%	95%	-	-
piperidine	0	0	10%	25%
N-ethylpiperidine	0	0	0	-
N-methylmorpholine	0	0	0	-
triethylamine	0	0	0	-

DBN can be seen to have an immediate effect on (*8*) causing rapid elimination. Secondary amines such as piperidine bring about elimination at a much slower rate whilst tertiary amines have little or no effect. In a second study the action of triethylamine on the cinnamate (*29*) in dichloromethane was found to produce no elimination products over a 15 hour period confirming the stability of the Bnpe-group to this base. Also, purification of Bnpeoc amino acids as their DCHA salts causes no elimination and leads to good yields of crystalline material. Table 2.3 summarises other bases and substrates investigated by a number of workers.

Table 2.3

BASE	Substrate	Comment	Ref.
pyridine (as solvent)	cinnamate (*29*)	stable 24 hr	3.7 V
imidazole/ DMF	Bnpeoc Ser (benzyl) OH	stable 18 hr	Gray[161b]
DMF/NH_3	benzyl-6, β-Bnpeoc OH-6 α-methyl penicillinate	stable 4 hr	Loyde[161c]
1 equiv. DMAP/DCM or pyridine	Bnpeoc-5'-o-thymidine	stable 24 hr	Turner[161d]
tetrabutyl ammonium fluoride	cinnamate (*29*)	rapid elimination	3.7 (IV)

In the case of tetrabutylammonium fluoride elimination was very rapid and associated with a blue colour. The fluoride ion is thought to be acting as a base to abstract the β-proton to leave the anion which is sufficiently stable to form an intermediate resembling Fig.1.36 giving rise to the colour.

Stability towards primary amines

As peptide bond formation involves the reaction of carboxyl-activated, Nα-protected amino acid with a primary amino group one might expect to observe the amine acting

as a base as well as a nucleophile. In the presence of base sensitive protecting groups this would lead to a mixture of products causing purification problems and reduced yields.

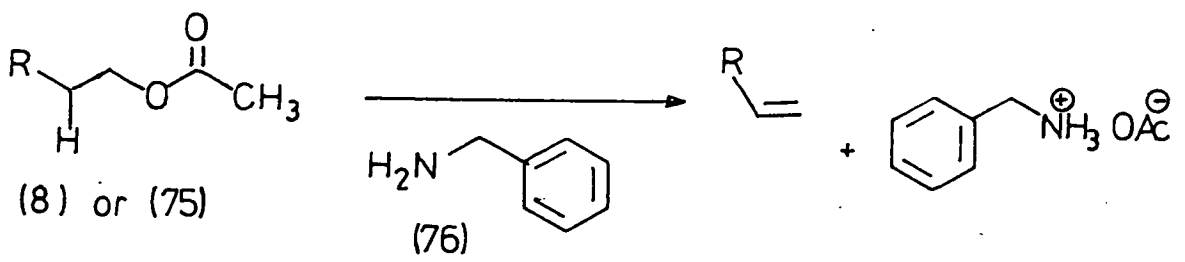
Table 2.4

Amine	pKa of conjugate acid	Amino acid	
triethylamine	10.8	H_2^+ GlyO$^\theta$	9.8
benzylamine	9.3		
NMM	7.4	H_2^+ LeuO$^\theta$	9.7
H-Gly OEt	7.6	H_2^+ PheO$^\theta$	9.2
H-Phe OMe	7.0		

The basicity of a peptide coupling reaction mixture is relatively low compared to reactions involving simple aliphatic amines as α-amino acid esters are relatively weak bases, as shown in Table 2.4[142]. However, Bodanszky and co-workers[97] found that Fmoc amino acids can be cleaved by primary as well as secondary and tertiary amines.

For our study we chose to investigate the action of benzylamine on the Bnpe and Fmoc acetates (*8*) and (*75*).

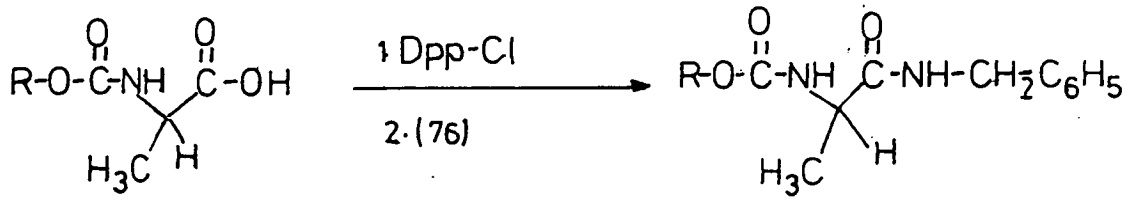
Fig.2.16



Benzylamine possesses a pKa similar to a free amino acid and would be a good model for studying the undesired β-elimination process. Addition of (*76*) was to a solution of the respective acetate in dichloromethane and the reaction monitored by H.P.L.C. It was found that up to 25% of the Fmoc acetate (*75*) had been consumed after five minutes and 35% after 2 hours. However the Bnpe acetate (*8*) was found to be intact under the same conditions. This study clearly shows the difference in lability between the two groups, with the implication that primary amines can act as a source for elimination of the Fmoc group which would lead to serious problems if repeated in peptide synthesis due to premature release of the α-amino function.

For our second study into the action of benzylamine, conditions more closely resembling a peptide coupling reaction were chosen. Benzylamine was added to an excess of carboxyl-activated Fmoc- or Bnpeoc-alanine (activation of carboxyl achieved with diphenyl phosphinic mixed anhydride) at 0°C. Stirring was continued for an hour at 0°C and then for an hour at room temperature.

Fig.2.17



R = Bnpe (44)
= 9-Fluorenylmethyl (77)

R = Bnpe (78)
= 9-Fluorenylmethyl (79)

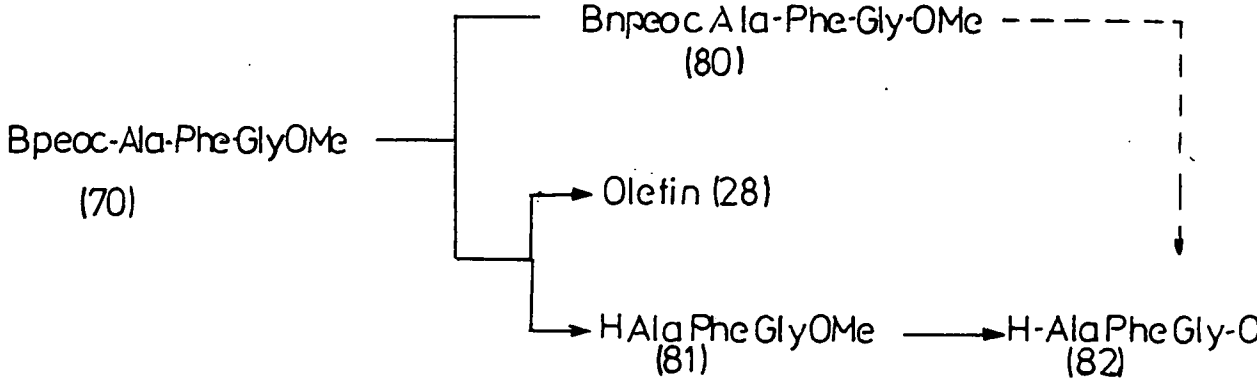
After this time the respective reactions were worked-up and the crude material obtained was analysed by H.P.L.C. for any

olefin formation. In neither case was any cleavage detected. These experiments show that, if excess acylating material is used with suitable activation, no base promoted elimination will occur during peptide synthesis.

Stability in presence of sodium hydroxide

Synthetic manipulations involving sodium hydroxide such as saponification, elimination, condensation and urethane formation make the degree of stability of the Bnpe group towards this alkali of considerable interest. As a useful synthetic study the saponification of the methyl ester Bnpeoc AlaPheGlyOMe (*70*) was attempted using 0.025M sodium hydroxide and dimethylformamide as the solvent. The reaction was worked-up after stirring for 1.5 hours to produce a foam, a t.l.c. of which indicated that about half the material had been saponified to the acid (*80*). As no ninhydrin active material was observed this indicates that very little amine (*81*) or (*82*) had been produced (see Fig.2.18).

Fig.2.18



The saponification of (*70*) was repeated with 4 equivalents of 0.05M sodium hydroxide, immediately on addition of which a blue colour formed which slowly faded to give a clear

solution. This blue colour must be due to the carbanion intermediate (c.f. Fig.1.36) and it can be concluded that these conditions are too strong. Production of the acid (*80*) in preference to the olefin (*28*) appears to rely upon using very dilute sodium hydroxide and on the relative amounts present. The solvent will also affect the course of reaction with a more polar solvent enhancing the general activity of the alkali. The acceptability of saponification in the presence of the Bnpeoc group has been demonstrated further illustrating the selectivity of Bnpe(oc) elimination.

2.3.3 Stability towards acids

For the purpose of peptide synthesis it is often desirable to remove protecting groups in the presence of other protected functions. As discussed earlier a scheme relying on differential acidolysis is prone to side-reactions and has led to the development of orthogonal protection. An obvious requirement therefore of a group designed to be selectively removed by base is that it should show absolute stability towards acid. Fig. 1.21 summarises the conditions used to remove the most commonly encountered acid-labile protecting groups. The Z-group is removed with HBr/acetic acid, Boc or ^{t}-butyl ester groups with TFA and the Dpp group with HCl-methanol.

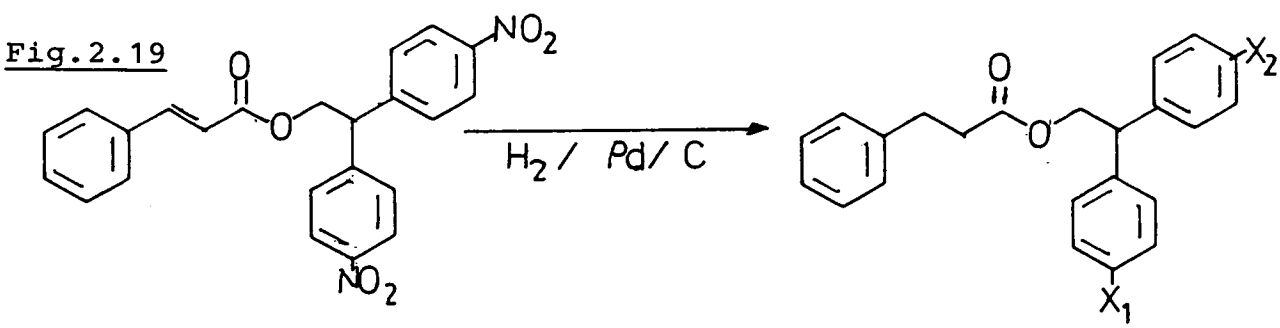
The cinnamate (*29*) was exposed to excess acid as indicated in Table 2.5.

Table 2.5

Acid	Compound	Comment	Ref.
TFA, 100%	cinnamate (*29*)	stable 24 hr	3.7 (iii)
3N HCl/MeOH 17 equivs.	cinnamate (*29*)	stable 18 hr	3.7 (iv)
HBr/acetic acid	Bnpeoc Lys (Z)-OH	stable	Ogunjabi[161e]

The results show the Bnpe(oc) group possesses excellent acid stability, a prerequisite for use in an orthogonal protection group strategy. An alternative method for removal of the Z-group is by catalytic hydrogenation over Pd/C. It was thus necessary to test the stability of the Bnpe cinnamate (*29*) to these conditions, see Fig.2.19. The reaction was stopped after 2 hours and examined by t.l.c. by which time two products of high polarity had formed. These products were ninhydrin active, implying that the nitro group had been reduced. The products were separated and identified by t.l.c., i.r. and n.m.r. From this analysis three products were shown to be present.

Fig.2.19



$X_1 = X_2 = NO_2$ (83) ; $X_1 = NO_2$, $X_2 = NH_2$ (84)

$X_1 = X_2 = NH_2$ (84)

The product of lowest polarity (coincident with the cinnamate (*29*) on t.l.c.) was found to be the dihydrocinnamate ester (*83*). The other two compounds of higher polarity were identified as the mono-amino and the diamino-dihydrocinnamates, (*84*) and (*85*) respectively. Production of these amino-compounds from (*83*) was after a reaction time of thirty minutes. Although more subtle conditions could probably be found to selectively hydrogenate the double bond of cinnamate (*29*) in the presence of the Bnpe(oc) group it would not be possible to remove the Z-group by catalytic hydrogenation in the presence of the Bnpe(oc) group.

2.3.4 Stability in solvents

Three Bnpeoc amino acids, Bnpeoc Val-OH (*56*), Bnpeoc Phe-OH (*52*) and Bnpeoc Trp-OH (*63*) were dissolved in either dichloromethane or dimethylformamide. Complete stability was observed in dichloromethane over 48 hours. However the acids in dimethylformamide showed about 4% cleavage after 24 hours and up to 16% after 48 hours. Bnpeoc amino acids have also been found to have limited stability in per-deuterated dimethylsulphoxide in which they decompose particularly rapidly when the acid functionality is masked as in dicyclohexylamino salts. Similarly, stability in dimethylformamide is reduced should the acid group be masked.

Lability in dimethylformamide is not restricted to the Bnpe(oc) group, Fmoc protected amino acids require that great care be taken to remove any traces of dimethylamine from

dimethylformamide either by distillation prior to use[143] or by changing solvents to dimethylacetamide. The advantages obtained from the discovery that Fmoc-derivatives have better long term stability in dimethylacetamide[144] can however be outweighed by the slower reactions relative to dimethylformamide. A comprehensive study of Bnpe(oc) stability in various solvents has been carried out by Gray[161b] who found in THF, dichloromethane and dimethylacetamide Bnpe(oc) has satisfactory stability properties. Crystalline Bnpeoc Ala-OH was found to be unaffected by storage under room conditions for many months.

2.4 ELIMINATION STUDIES

2.4.1 Introduction

The stability studies in Section 2.3.1 demonstrated the unique qualities possessed by DBN to effect a rapid elimination of the Bnpe(oc) group. It was therefore decided to investigate the conditions affecting DBN promoted elimination.

2.4.2 Factors affecting elimination

The first factor investigated was the effect of temperature on the rate of elimination. It was also hoped that this study might produce some information about the mechanism of elimination. The cinnamate (*29*) in 25 ml dichloromethane was equilibrated at various temperatures set by a thermostatted bath and 1 equivalent of DBN was added at which time a stopwatch was started and the temperature of the reaction liquid recorded. At appropriate intervals aliquots were withdrawn to which were added an excess of 1M acetic acid in methanol. After work-up this solution was analysed by H.P.L.C. to provide a quantitative record of the cinnamate (*29*) peak disappearance. The H.P.L.C. integrator had previously been calibrated against standard samples of acetate (*8*), cinnamate (*29*) and olefin (*28*) (see Appendix A, 1-3).

Elimination of the cinnamate (*29*) with 1 equivalent of DBN was achieved at 18°C, 8°C, -3°C and -17°C. From a qualitative perspective the reaction time for complete elimination was found to increase as the temperature was decreased, as would be expected, see Table 2.6.

Table 2.6

Temperature	Time for elimination
18°C	42 min
8°C	122 min
-3°C	205 min
-17°C	285 min

As the cinnamate (*29*) peak height on the H.P.L.C. trace is directly proportional to its concentration (see Appendix A, Fig.1), a plot of Ln of 1/ester peak area would show whether the overall reaction kinetics were first or second order. The figures in Appendix A, Fig.4-11, represent plots of Ln and 1/peak area against time at 18°C, 8°C, -3°C, -17°C. The best fit line represents the rate of elimination (k) and together with the standard deviation summarised below in Tables 2.7 and 2.8.

Table 2.7

Results using Ln (ester peak area) against time (1st order plot)

Temperature	Gradient, K, s^{-1} ($x10^4$)	Standard deviation	No.of point
18°C	-9.9	±1.2 (12%)	11
8°C	-2.76	±0.13 (5%)	9
-3°C	-1.76	±0.17 (10%)	9
-17°C	-0.94	±0.08 (9%)	15

Table 2.8

Results using 1/ester peak area against time (2nd order plot)

Temperature	Gradient, k, $mol^{-1}s^{-1}$ ($x10^7$)	Standard deviation	No.of point
18°C	2.0	±0.4 (20%)	11
8°C	0.53	±0.06 (11%)	9
-3°C	0.21	±0.04 (19%)	9
-17°C	0.14	±0.015 (11%)	15

From Tables 2.7 and 2.8 and Figures 4-11 in Appendix A, the results show that in all cases the data are more consistent with a first order elimination than second order as indicated by the closer approximation to a straight line plot for Ln ester peak area against time with respect to 1/ester peak area against time.

From the discussion of the mechanisms for elimination in Section 1.3, a summary of which is given in Table 2.9,

(E1) anion was the only mechanism discussed that should show first order kinetics,

Table 2.9

Mechanism	Order
(E1) anion	1st
$(ElcB)_R$	2nd
$(ElcB)_{IP}$	2nd
$(ElcB)_{IR}$	2nd
E2	2nd

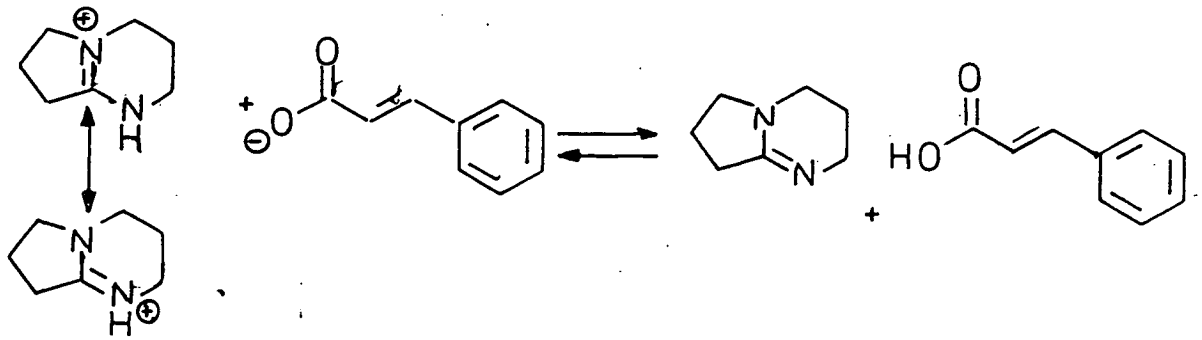
Elimination through this mechanism would be independent of further base addition providing one equivalent was present. In order to confirm which of these mechanisms was present, a further set of kinetic experiments was performed using excess base. The experiments were carried out at -5°C as described above with 2 equivalents of DBN then the procedure was repeated with 4 equivalents of DBN, the results are shown below in Table 2.10.

Table 2.10

No.of equivs DBN	Rate at -5°C $(x10^4)$ s^{-1}
2	1.8
4	4.5

The rate can be seen to double as the amount of base is doubled showing unambiguously that the elimination mechanism is not (E1) anion. However the first set of kinetic experiments implied a first order rate. As all the possible mechanisms in Table 2.9 rely on both substrate and base concentration [other than (E1) anion], then there must be some other mechanism present. This can be explained if these reactions were occurring in a pseudo first order fashion. This could happen if the base was continually being regenerated as was the case with $(CN)_2CHC(CN)_2NHC_6H_3Me_3$ (eqn.1.5, Section 1.3).

Equation 2.1



This equilibrium would be expected to lie towards the L.H.S., however sufficient DBN might be produced to explain the apparent first order kinetics.

To obtain the activation energy from these rates involves plotting the Ln of the rates against 1/temperature °K in an Arrhenius graph so that $k = Ae^{-E_A/RT}$

$$Lnk = LnA - E_A/RT$$

where the gradient $m = - E_A/R$

Table 2.11

1/temperature	Ln (rate for 1st order)	Ln (rate for 2nd order)
3.44×10^{-3}	-6.9	-15.42
3.56×10^{-3}	-8.20	-16.76
3.70×10^{-3}	-8.64	-16.37
3.90×10^{-3}	-9.27	-18.13

Gradient obtained for Arrhenius plot (Lnm for 1st order)

$m = -4470 \pm 972$

$\therefore E_A = 37 kJmol^{-1} \pm 8 kJmol^{-1}$

Gradient obtained for Arrhenius plot (Lnm for 2nd order)

$m = 4979 \pm 1091$

$\therefore E_A = 41 kJmol^{-1} \pm 9 kJmol^{-1}$

See Figs. 14 and 15, Appendix A.

The relative size of the activation energy obtained for the elimination of 1,1-bis(4-nitrophenyl)ethene (*28*) from (*29*) cannot be directly compared with other activation energies for similar compounds where the studies have been done using alkoxide bases in aqueous systems. The activation energies obtained for the later compounds under the conditions mentioned are around $80-100 kJmol^{-1}$ [62].

From the results obtained it is impossible to determine whether the mechanism is a two-step (ElcB) reaction or an extreme form of E2 elimination. Differentiation between

these mechanisms involves measuring (1) the extent of transfer of the β-proton by using β-deuterium or β-tritium isotope effect, (2) the extent of bond weakening of the leaving group using α-carbon isotope effect, or (3) measuring the driving force of the reactions since the concerted path to be utilized should be of a lower energy, and hence faster than the possible stepwise path.

The proposed mechanism for base 'renewed', eqn.2.1 requires that the rate of base 're-newal' be greater than the rate at which the base is removed during elimination. This being so, it was thought to be of interest to investigate the action of excess acid on this equilibrium. If the equilibrium is still sufficient to supply base at such a rate that elimination is not affected, or slowed down to a significant extent, then deprotection can be brought about in such a way that only the acidic β-proton is effectively exposed to the free base, decreasing the likelihood of unwanted side reactions. With addition of acetic acid to buffer DBN it was hoped to obtain exceptionally mild and selective deprotection conditions.

The results in Table 2.12 were obtained with the cinnamate (*29*) dissolved in either dichloromethane or dimethylformamide. to which was added the required quantity of acetic acid followed by an equivalent of DBN and the reaction was monitored by H.P.L.C. as described previously.

Table 2.12

Results using (*29*) with DBN/HOAc

No.of equivs DBN	No.of equivs AcOH	Solvent	Elimination time	Ref
1	0	CH_2Cl_2	40 min	3.8I
1	1	CH_2Cl_2	>2 hrs	3.8II
2	1	CH_2Cl_2	60 min	3.8III
1	1	DMF	∿15 min	3.8IV
1	2	DMF	30 min	3.8V
1	4	DMF	60 min	3.8VI
1	8	DMF	120 min	3.8VII
1	TFA	DMF	>24 hrs	3.8VIII

The use of 1 equivalent of acetic acid in dichloromethane drastically reduces the rate of elimination (3.8II, Table 2.12). However if the solvent is changed to dimethylformamide elimination proceeds very efficiently (3.8IV, Table 2.12). Addition of 2 equivalents of acetic acid roughly doubles the time for elimination and thereafter doubling the number of equivalents of acetic acid doubles the elimination time. Addition of 1 equivalent of TFA (3.8VIII,Table 2.12) effectively stops elimination as substantial amounts of cinnamate (*29*) were present after 24 hrs reaction with DBN/TFA. These results also show a remarkable solvent effect on the efficiency of elimination as shown by comparison between 3.8II and 3.8IV in Table 2.12. A study into the effect of solvent change on elimination was carried out using the tripeptide, Bnpeoc AlaPheGlyOMe (*70*) (7.4 mMolar) and the

reaction followed as previously described with H.P.L.C., the results given in Table 2.13.

Table 2.13

Effect on elimination of (*70*) with DBN on changing solvent

Base	Solvent	No.equivs AcOH	Molarity	Elimination time
DBN	CH_2Cl_2	0	$7.35x10^{-3}M$	3 hrs
DBN	DMF	0	$7.4x10^{-3}M$	30 min
DBN	DMF	1	$7.4x10^{-3}M$	30 min

From the results in Tables 2.12 and 2.13 there can be identified two related factors working in opposition. The first factor is demonstrated by the solvent change from dichloromethane to dimethylformamide producing a greatly increased rate of elimination. This could be attributed to increased solvation of a charged intermediate (for an ElcB mechanism) and improved solvation of the product (acid-anion) (see Section 1.3). The addition of acetic acid produces an opposite effect. In dichloromethane the rate of elimination is reduced to an unacceptable level for general use. This may be due to the base recycle equilibrium shown in eqn.2.1 lying too far to the L.H.S. on addition of acetic acid so that the equilibrium is no longer capable of supplying free base at a rate required for rapid elimination to occur in dichloromethane. The two mechanisms can be seen to be dependent on one another, so that break down of one will halt the catalytic nature of the elimination and therefore retard the overall reaction. However, when

dimethylformamide is used both mechanisms must be favourably influenced as no decrease in elimination rate is observed on addition of an equivalent of acetic acid to the reaction mixture with DBN and the tripeptide (*70*) shown in Table 2.13.

The results from these series of experiments indicate the mechanism of elimination is probably via a polar intermediate (ElcB), and the addition of an equivalent of acetic acid with DBN has little affect on the rate of elimination. The combination of DBN and acetic acid in dimethylformamide would allow conditions to be tailored to buffer against any possible occurrence of side reactions due to the presence of a powerful free base (DBN). The efficiency of elimination would remain unchanged providing exceptionally mild and selective deprotection conditions. The pKa of the acid used to buffer the base is of obvious importance as demonstrated by the addition of TFA (Table 2.12) which effectively stops elimination. A second example demonstrating the importance of the buffering acid was found as deprotection of Bnpeoc Val (*56*) in dichloromethane is not obtained until two equivalents of DBN are added. The pKa's of TFA, Bnpeoc Val, cinnamic acid and acetic acid are approximately 0.3, 2.5, 4.4 and 4.75 respectively. From this series has been shown that if an acid is present with a pKa less than 4.4 the rate of elimination will be seriously retarded.

Base and substrate molarity

To investigate the effect of the base used and the substrate molarity on the rate of elimination the tripeptide,

Bnpeoc AlaPheGlyOMe (*70*) was dissolved in solvent to which was added either DBN or DBU. The reactions were monitored as described previously with H.P.L.C.

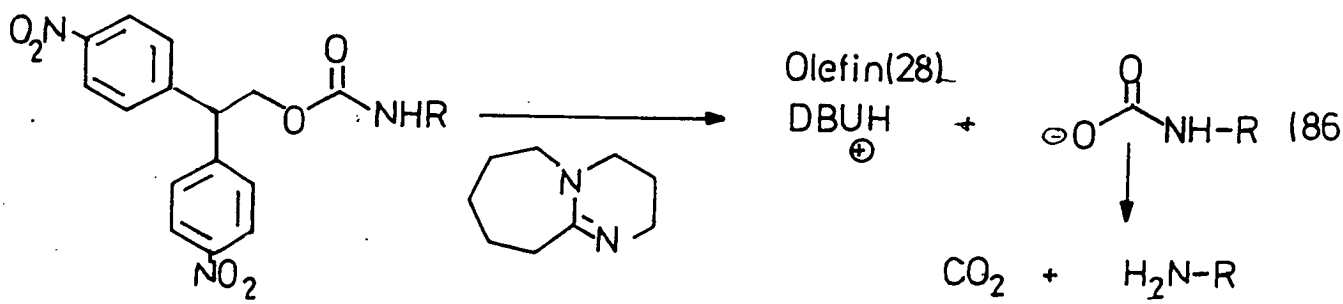
Table 2.14

Base	No.of equivs base	Solvent and molarity	Time for elimination
DBN	1	CH_2Cl_2 7.4mM	3 hrs
DBN	2	CH_2Cl_2 7.4mM	1.5 hrs
DBN	1	DMF 7.4mM	30 min
DBU	1	DMF 7.7mM	20 min
DBN	2/2 HOAc	DMF 234mM	<10 min

The results in Table 2.14 demonstrate that doubling the amount of base used effectively halves the elimination time, confirming the result obtained with cinnamate (*29*) in Table 2.10. The results also show that the rate of reaction is directly related to the substrate molarity as the elimination time is decreased to below ten minutes on increasing the molarity. The rate of reaction is also increased when using DBU rather than DBN, with elimination complete in 20 rather than 30 minutes. During the course of this reaction

a transient intermediate was produced which was more polar than the starting material. This was thought to be the carbamic acid (*86*), see Fig.2.20.

Fig.2.20



With the addition of acetic acid this system would provide a buffered environment which would encourage the decomposition of the carbamic acid by protonation.

From this section the factors affecting elimination can be summarised as follows.

(1) Polarity of solvent.

(2) Substrate concentration.

(3) Number of equivalents of base.

(4) Temperature.

(5) Base used; either DBN or DBU.

(6) Number of equivalents acetic acid used.

A fast and efficient deprotection will be obtained if factors (1)-(4) are increased and DBU is used rather than DBN with the number of equivalents of acetic acid used no more than one.

2.4.3 Comparison of elimination conditions

As discussed in Section 1.6, the Bnpe(oc) group has

structural similarities to the Fm(oc) and p-nitrophenylethyl(oxycarbonyl) groups. We therefore thought it would be of interest to investigate the relative lability of these groups under similar conditions. For this purpose p-nitrophenylethyl cinnamate (*31*) was synthesised under standard conditions described in Section 2.2.2. This compound was dissolved in dichloromethane to which was added an equivalent of DBN. The reaction was monitored by H.P.L.C. and no elimination was observed. Under the same conditions elimination of Bnpe cinnamate (*29*) was complete after forty-five minutes. The experiment was repeated with cinnamate (*31*) and a vast excess of 1 molar DBN in pyridine as described by Pfleiderer for his deprotection conditions;[26] this brought about rapid elimination in less than ten minutes.

The second set of comparative reactions carried out used the Fmoc alanine benzylamide (*79*) previously synthesised for tests on lability in the presence of primary amines (Section 2.3.2). To a solution of (*79*) in dimethylformamide was added an equivalent of DBN which brought about complete elimination within ten minutes. This experiment was repeated with an equivalent of DBN and two equivalents of acetic acid; elimination was complete after forty-five minutes cf. Table 2.12. Removal of the Fmoc-group as described by Carpino and Han[28] and subsequently used by Sheppard and co-workers[29] is normally achieved with a vast excess of a 20% solution of piperidine in DMF. With analogy to the results obtained for removal of Fmoc from (*79*) with DBN/acetic acid then deprotection of the Bnpe(oc) group should be possible with a 20% piperidine solution. In fact elimination of (*29*)

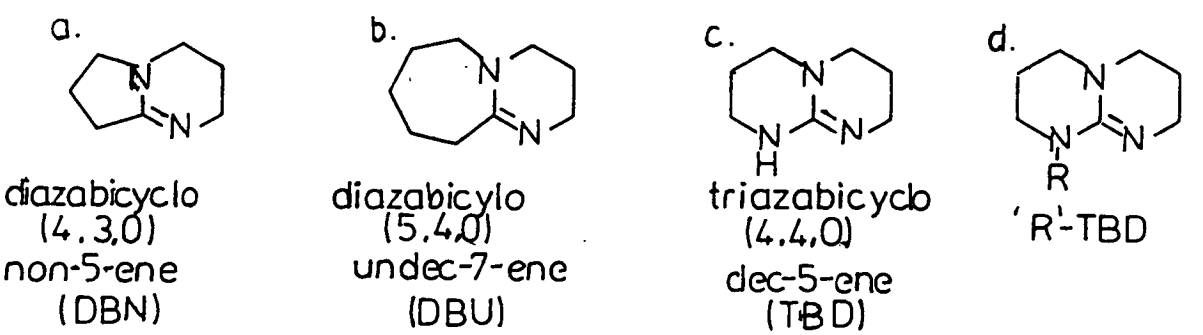
was complete after 15-20 minutes with a 20% solution of piperidine and acetic acid in dichloromethane.

These experiments show that the conditions developed for the removal of the Bnpe(oc) group can be used for Fmoc deprotection whilst the p-nitrophenylethyl group is unaffected. The advantage provided with the use of Bnpe(oc) deprotection conditions can be seen by the near stoichiometric amounts of base required in contrast to the deprotection strategies currently used for Fmoc and p-nitrophenylethyl(oxycarbonyl) groups which rely on using vast excess of base and so incur the possible risk of damage to the protected substrate.

2.4.4 Polymer supported base elimination

A polymer supported base for the removal of the Fmoc group has been used[145,146] and this approach was thought to be useful for Bnpe(oc) group deprotection.

Fig.2.21



The bases DBN and DBU used in the elimination study belong to a family of bases some of which are shown in Fig. 2.21. In this series, base (d) can have R as methyl or polystyrene resin. To investigate the potency of this reagent to remove the Bnpe(oc) group, 1 equivalent was added to a dichloromethane solution of Bnpe cinnamate (*29*) and allowed to stir for twenty-four hours. After work-up of the reaction

the olefin (*28*) was isolated from the organic phase whilst most of the acid was found to have remained on the resin and was only removed after washing the resin with 90% acetic acid. This experiment was repeated with 10 equivalents TBD-resin in dichloromethane and elimination was found to be complete after thirty minutes. Deprotection of the Bnpeoc AlaPheGlyOMe (*70*) with 13 equivalents TBD-resin was complete after 24 hours as indicated by H.P.L.C. The carbamic acid intermediate (*86*) was liberated from the resin and decarboxylated with acetic acid water (9:1). These results provide an obvious opportunity to create a flow system whereby deprotection is effected by simply passing a solution of the substrate through a small column of polymer bound base. Overall contact time of substrate to base would be short and the products recovered in solution free from base. Additionally, acetic acid could be added to ensure the deprotected species remains in solution and not as an ion-pair on the resin.

2.4.5 Reactivity of 1,1-bis(4-nitrophenyl)ethene (*28*)

Removal of the Bnpe(oc) group produces the deprotected functionality and the olefin (*28*). This olefin is required to be chemically inert under the deprotection conditions used. Should this olefin not be of suitable stability under these conditions then by-products could be obtained, such as reaction with the deprotected functionality or with the base used for deprotection. The latter feature is a characteristic of the deprotection conditioning used for Fmoc removal with 20% piperidine in vast excess. In this case dibenzofulvene reacts with the excess cyclic amine. This phenomenon has

been used to remove dibenzofulvene from a deprotection reaction with excess polymer supported base but with only limited success[145,146].

For our studies we investigated the reaction of (*28*) with cinnamic acid (*30*) under various conditions. The acid (*30*) and olefin (*28*) were stirred or refluxed with boron trifluoride etherate in dichloromethane; p-toluene sulphonic acid in dichloromethane or toluene; or methanolic hydrochloric acid. In all cases no reaction was observed on varying the relative amounts used, changing the solvent or addition of excess Lewis acid. It would appear from these results that olefin (*28*) is particularly stable towards nucleophilic attack. With the deprotection conditions developed in this section no by-products have been observed although with analogy to Fmoc some reactivity might be expected under certain conditions. The choice of deprotection conditions is therefore of prime importance in the development of a successful protecting group in order to avoid any possibility of olefin reactivity.

2.5 SOLID PHASE PEPTIDE CHEMISTRY

In contrast to the deprotection strategy proposed in Section 2.4.4 the substrate rather than the reagent can be attached to the resin so that synthetic manipulations can be carried out on the solid support and the reagents removed by filtration. This was the concept introduced to peptide synthesis by Merrifield as discussed in Section 1.4.3.

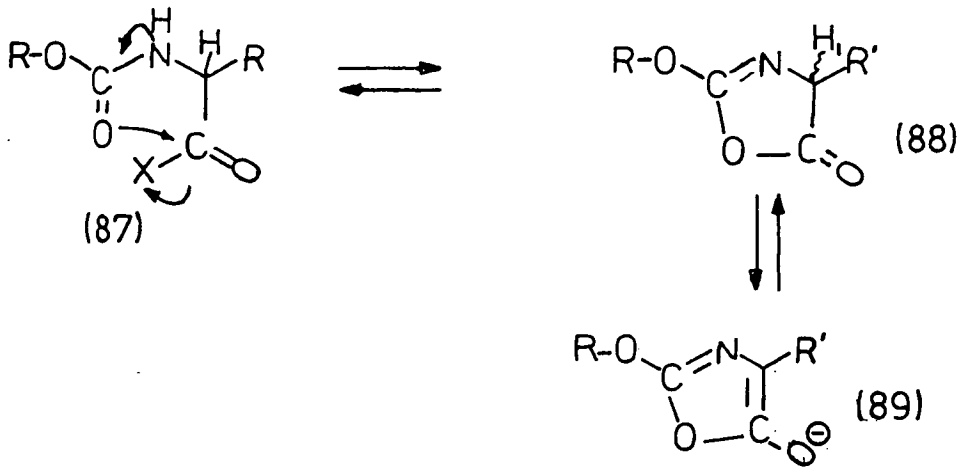
One of the most critical steps in a solid phase synthesis is the attachment of the first residue onto the functionalised resin. Should this step not proceed efficiently and cleanly then the whole synthesis will be jeopardized. As discussed in Section 1.4.4 the advantages offered using the p-alkoxybenzyl alcohol resin in combination with an orthogonal protection stragegy have allowed this type of resin to become one of the most widely used in solid phase peptide synthesis.

The standard procedure for attachment of the first residue onto this resin is by addition of excess symmetrical anhydride, obtained by reaction with DCCI, to the resin in the presence of 1 equivalent of DMAP[89]. However this procedure suffers from two major drawbacks, racemisation and dimer formation.

2.5.1 Racemisation of protected amino acids and ester formation to p-alkoxybenzylalcohol resin

Racemisation is associated with oxazolone formation by the mechanism set out in Fig.2.22.

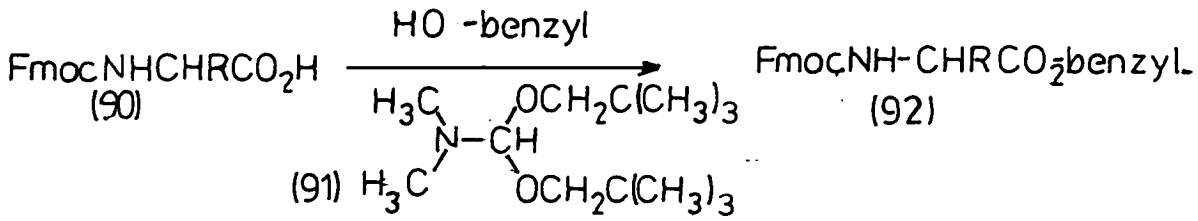
Fig.2.22



Compound (*88*) can racemise faster than it is attacked by a nucleophile such as an amine group. The observation that oxazalone formation can occur with alkoxycarbonyl protected amino acids[147] using carbodiimide type condensing reagents disproved the long held belief that such protection groups were immune to this cyclisation. The ease with which the acidic proton (H_1) can be removed by a base from the chiral centre is of particular significance when DMAP is added to the symmetrical anhydride to accelerate slow couplings[148] or used to couple the first amino acid onto the resin. DMAP is a very powerful base and up to 10.5% racemisation[149] is observed when coupling alanine, phenylalanine or valine as their symmetrical anhydrides to the resin in the presence of 1 equivalent DMAP. This racemisation can be reduced if only a catalytic amount of DMAP, along with 1 equivalent of N-methyl-morpholine, is used for coupling in dimethylformamide[150]. Other methods for ester formation include condensation with

DCCI/DMAP[151], active esters in the presence of imidazole[152] or N-N-dimethylformamide dineopentylacetal (*91*) as activating intermediates[153].

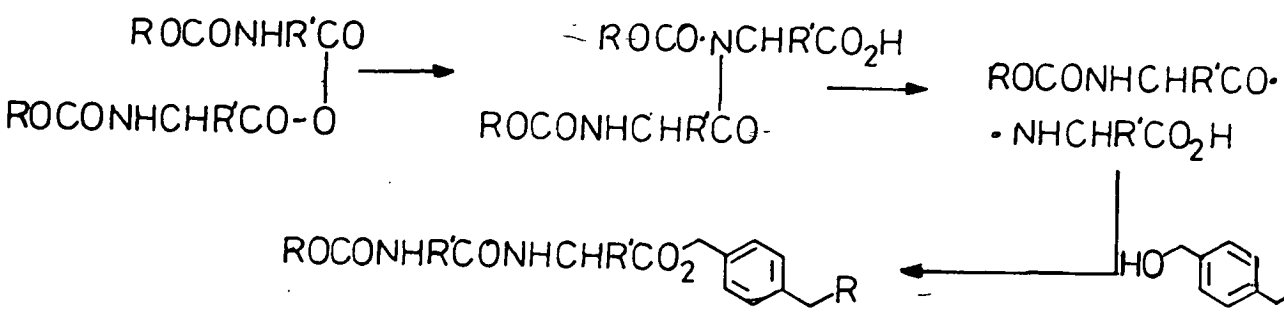
Fig.2.23



These methods have all been associated with racemisation, long reaction times or relatively low esterification.

The second problem encountered with the use of DMAP to enhance esterification of the resin is the formation of dimers. When anchoring FmocGly to the resin Pedroso and co-workers observed as much as 9% GlyGly formation[154]. This can be explained by the lability of Fmoc in the presence of DMAP which has led to Atherton and Sheppard recommending that a (base stable) Boc amino acid be attached as the first residue[95]. An alternative mechanism to account for dimer formation has been described by Merrifield[155]. In this mechanism the first step is disproportionation of a mixed anhydride to form the symmetrical anhydride followed by intramolecular N-urethane acylation promoted by the presence of tertiary amine salt.

Fig.2.24



The method of ester formation we have investigated was developed from the recent revival in interest in the use of acid-chlorides[156,157]. Fmoc and Bnpeoc acid chlorides, prepared by the method of Carpino[156], were added to p-alkoxybenzyl alcohol resin swollen in the required solvent with the reagents summarised in Table 2.15. Valine and glycine were chosen, as these residues would provide a stringent test of the method with respect to its susceptability to both racemisation and dimer formation. The results obtained for valine which possesses a sterically bulky residue, and for glycine, which has no side-chain, provide a basis to judge the efficiency of ester formation.

Table 2.15

Esterification using 4Bnpeoc Val-Cl (*93*) DCM stirred with DMAP and/or 3° amine

Base	DMAP/mol (*93*)	Esterification	Oxazolone formation i.r.
2NMM	0	0	1840, 1680 cm^{-1}
2NMM	1%	v.low	1840, 1680 cm^{-1}
2NMM	5%	49%	-
2,6-Lutidine	5%	31%	-

In order to gauge the efficiency of the acid chlorides with respect to the standard conditions, a resin sample was esterified using 2 equivalents of Bnpeoc Val symmetrical anhydride (*95*) in the presence of 20% DMAP. This produced

a resin with 47% esterification cf. Table 2.15.

The percentage esterification was calculated from the elemental analysis of the p-alkoxybenzyl resin using the formula:

$$F_R = \frac{\%N}{x.100-\%N.M_w}$$

x = No.of N's present x 14

M_w = molecular weight of added group on resin

F_R = (mmol./g functionalisation of resin).% esterification

This formula takes into account the non-linearity of the variation of nitrogen with esterification.

The results in Table 2.15 show that in the absence of DMAP, cyclisation to the oxazolone is favoured and no esterification is obtained. With 1% DMAP oxazolone formation is still predominant with only a very small amount of ester formation. However, increasing to 5% DMAP brings about a complete change in reaction. Ester formation proceeds with an efficiency equal to that obtained using symmetrical anhydride/20% DMAP and no oxazolone formation was observed by i.r. of the solution. The possibility of racemisation *via* oxazolone acylation of the resin can be discounted as no esterification was observed with a reaction mixture containing predominantly oxazolone (i.r., 1680 and 1840 cm^{-1}). Increasing the percentage of DMAP increased esterification in such a way that it avoided oxazolone formation as indicated by the lack of characteristic peaks at 1840 and 1680 cm^{-1} in the reaction fluid.

From this model study (see Table 2.15) the conditions which gave best results were achieved with 5% (mole/mole acid chloride) DMAP with 2 equivalents of N-methylmorpholine as shown by the efficient esterification of the resin without oxazolone formation. Using these results a synthesis of Bnpeoc amino acid resins was attempted under preparative methods used for solid phase peptide synthesis whereby mixing is achieved by a rotary agitator rather than stirring which could damage the resin beads. For these experiments 1.5 equivalents of acid chloride were added to the resin in dichloromethane containing 5% DMAP and 2 equivalents N-methylmorpholine and shaken for 30 minutes. After draining off the reagents the resin was swollen in dimethylformamide and a further equivalent of acid chloride was added to the reaction mixture.

From this study (Table 2.16) a remarkable difference in ester forming ability of Bnpeoc Gly (*94*) with respect to Bnpeoc Val (*93*) was obtained as shown by the lack of ester formation for Bnpeoc Val in Table 2.16. This difference could be attributed to the inability to obtain conditions that combine the high concentration of reagents required for optimum ester formation with the low viscosity required for mechanical shaking. The latter feature requires the reaction vessel to be about half-full. This can cause diffusion of the reagents out of the reaction vessel to the 'dead-volume' below the sintered filter, effectively removing them from the reaction mixture. The steric difference between the two amino acids is also reflected in Table 2.16 by the generally lower % esterification obtained with Bnpeoc Val-Cl.

Table 2.16

Results of acylation with rotary shaking/5% DMAP (Resins (*96*) and (*97*))

Reagent		Esterification
(i) 1.5 Bnpeoc Val-Cl	CH_2Cl_2/shaker	18%
1 " "	DMF/shaker	22%
(ii) 1.5 Bnpeoc Gly-Cl	CH_2Cl_2/shaker	44%
1 " "	DMF/shaker	81%

To overcome these problems esterification was repeated using the same amount of DMAP and N-methylmorpholine in the minimum amount of solvent with Bnpeoc Val-Cl (*93*), (as an example of the worst possible case) and difficulties concerning mixing overcome by placing the reaction vessel in a sonic bath.

Table 2.17

A comparison of esterification with shaking and sonication of resins (*96*) and (*98*)

Reagent		Esterification
(i) 2.5 Bnpeoc Val-Cl	CH_2Cl_2 / DMF) shaker	22%
(ii) 1.5 Bnpeoc Val-Cl	CH_2Cl_2/sonic	40%
1 Bnpeoc Val-Cl	DMF/sonic	47%

A considerable increase, from 22% to 44%, in esterification of the resin with Bnpeoc Val-Cl was obtained using the sonic bath method (Table 2.17). Together with these results the results in Table 2.15 must be considered which show that 4 equivalents of Bnpeoc Val-Cl are required to bring about the same amount of esterification as achieved by 1.5 equivalents of Bnpeoc Val-Cl and sonication. From these experiments it can be concluded that the conditions provided by agitating the reaction mixture in a minimum amount of solvent by sonication is a considerable improvement on either rotary shaking or mechanical stirring.

Racemisation studies for esterification with 5% DMAP

The possibility of racemisation was investigated using the modified Manning and Moore technique[158,159]. The deprotected valine resins were coupled with Boc Ala/OH using DCCI in dichloromethane. Following work-up the dipeptides were cleaved from the resin with TFA and a sample was taken for analysis on an ion-exchange column of an amino acid analyser. Samples of DL and LL dipeptides were independently prepared and used to standardise the ion-exchange column.

The results obtained from the valine substituted resins prepared by treatment with Bnpeoc Val-Cl (*93*) and 5% DMAP with sonication are given below, together with the standards. In all cases the ion-exchange column length was 0.6 x 24 cm at 75°C and the samples dissolved in a 0.2M sodium citrate buffer, pH 3.49.

Table 2.18

Retention times for LL and LD AlaVal dipeptides obtained in racemisation studies

Sample	L.D. dipeptide (min)	L.L. dipeptide (min)	Ref
Ala (D.L.) Val	67	71	Appendix C Fig.1
Ala (L) Val	- (<0.04%)	72	Appendix C Fig.2
Ala Val (Bnpeoc-resin) (98)	- (<0.04%)	73	Appendix C Fig.3

Table 2.18 shows that the diastereoisomers Ala (D) Val (L) and Ala (L) Val (L) are separated on ion-exchange with retention of 67 and 71 minutes. The standard Ala (L) Val (L) confirms the second peak at 71 minutes to be the chirally pure material. When no peak was recorded on the ion-exchange chromatogram the sensitivity of the result is given in parenthesis, as defined by the minimum threshold value required to activate the integrator in the amino acid analyser.

Using these standards for comparison, dipeptide from resin (98) produced only one peak which corresponded to chirally pure AlaVal dipeptide with less than 0.04% racemisation.

The significance of this result is substantial as the contemporary methods generally used for direct ester formation are all associated with problems of racemisation.

As no racemisation is observed for the AlaVal dipeptide derived from Bnpeoc Val-Cl then it can be concluded that protection with Bnpeoc ONSu and deprotection with DBU/ acetic acid also involves no racemisation. Independent data from $[\alpha]_D$ measurements and circular diachroism spectra (Appendix D) also provide proof of chirality of Bnpeoc amino acids.

2.5.2 Dimer Formation

Deprotection of Bnpeoc Gly-resin was accomplished with DBU/acetic acid and the glycine washed from the resin with TFA in dichloromethane. The chromatograms obtained from Fmoc[162] and Bnpeoc resins are shown in Appendix C and results given below, see Table 2.20.

Table 2.19

Table of retention times for the analysis of GlyGly dimer content on resins prepared with 5% DMAP and 2.5 Bnpeoc or Fmoc-Gly

	Gly min	GlyGly min	dimer content
Bnpeoc Gly-resin (*97*)	19	46	6%
Fmoc Gly-resin (*99*)	19	46	5%

Standard samples of Gly and GlyGly gave retention times of 19 and 46 mins respectively. These results (Table 2.19) illustrated in Appendix C, Figs. 8 and 9 show that dimer formation accounts for about 5% of the total glycine content of either Fmoc or Bnpeoc resins. As discussed previously

two possible mechanisms have been proposed for the formation of dimers, either lability of N-protecting group or N-urethane acylation. In order to reduce the possibility of the former, esterification was repeated with pyridine in dichloromethane as the solvent, with no DMAP, whilst to reduce the possibility of the latter an equivalent of acylating reagent was used. To judge the effectiveness of this new method an equivalent of acid chloride was added to the resin mixture containing 5% DMAP in methylmorpholine in dichloromethane.

Table 2.20

	1 Bnpeoc Gly-Cl NMM/5% DMAP (*100*)	1 Bnpeoc Gly-Cl pyridine (*101*)	1 Fmoc Gly-Cl pyridine (*102*)
esterification	22%	88%	75%
dimer content	<0.7%	<0.2%	<0.2%

The results in Table 2.20 show the predictable lack of ester formation on using only one equivalent of acid chloride with 5% DMAP, cf. Table 2.16. However the remarkable ability for ester-formation promoted by pyridine in dichloromethane provides a much improved method. In both examples when using one equivalent of acylating reagent with either DMAP or pyridine no GlyGly dimer formation was observed as indicated by amino acid chromatography (Appendix C, Fig. 10 and 11). The chemical integrity of the glycine resin, obtained in excellent yield by this route, together with the ease of preparation using only one equivalent of acylating agent is a substantial improvement over the other methods discussed.

The opportunity to exploit this method for general resin-ester bond formation was then investigated with Bnpeoc Val and Fmoc Val. Table 2.21 gives the results obtained when either Bnpeoc- or Fmoc-valine acid chloride were allowed to sonicate with p-alkoxybenzyl alcohol resin and pyridine in dichloromethane for 2 hours.

Table 2.21

Resin esterification using 1 or 1.1 equiv. Bnpeoc- or Fmoc-valine acid chloride/pyridine to give resins (103), (104) and (105).

	Reagent	Esterification	Racemisation
1	Bnpeoc Val-Cl	58%	<0.08%
1.1	Bnpeoc Val-Cl	74%	<0.06%
1.1	Fmoc Val-Cl	60%	<0.09%

As could be predicted slightly less esterification was obtained relative to glycine. No racemisation (<0.09%) was observed and when a 10% excess of acid chloride is used with respect to the resin functionalisation the esterification of sterically bulky amino acids can be considerably improved, from 58% to 74%.

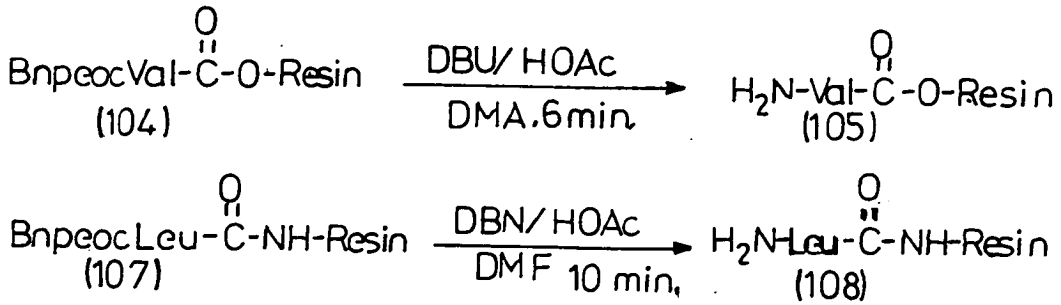
In conclusion, the results from this section have demonstrated the ability to overcome two problems associated with the anchoring of the first residue, which are often encountered but not always recorded. The solution proposed provides a highly efficient and mild

procedure which maintains the chemical integrity of the residue both in terms of chiral purity and prevention of dimer formation. The cost efficiency is a substantial improvement on other methods which rely on using four times as much material with less satisfactory results.

2.6 DEPROTECTION STUDIES ON SOLID PHASE

The method for removal of the Bnpeoc group developed in solution phase can equally well be applied to solid phase synthesis. This has been demonstrated by monitoring the deprotection of Bnpeoc valine p-alkoxybenzyl ester (*104*) with 1.2 equivalents of 0.3 molar DBU in dimethylacetamide together with acetic acid. The conditions used were those recommended during the deprotection cycle on a commercial solid phase peptide synthesis† save that the resin was agitated using a sonic bath. Complete deprotection of the Bnpeoc group was recorded after 6 minutes and confirmation of no further olefin formation was obtained by washing the resin thoroughly and adding a fresh quantity of DBU and acetic acid in dimethylacetamide. A second deprotection study used 1.2 equivalents of DBN and acetic acid in dimethylformamide and a manual rotary shaker. This study was also monitored by H.P.L.C. which indicated complete deprotection after ten minutes. Confirmation of no further olefin formation was obtained as above. These studies categorically prove the ease of removal of the Bnpeoc group under mild and selective conditions with DBU or DBN and acetic acid in either dimethylformamide or dimethylacetamide, see Fig.2.25.

Fig.2.25



† Applied Biosystems model

2.7 PEPTIDE SYNTHESIS

The general method for solid phase peptide synthesis outlined in Sections 1.4.3 and 1.4.4 requires that the first amino acid is anchored to the resin followed by removal of the Nα-protecting group. The (n-1) Nα-protected, carboxyl-activated amino acid is then coupled, and this cycle repeated with as many protected amino acids as required for the finished sequence. The anchoring and deprotection have been dealt with in Sections 2.5 and 2.6, and the synthesis of the protected amino acids in Section 2.2.4. All these steps can be combined into an automated solid phase peptide cycle with Bnpeoc amino acids.

To date this area is still at a very early stage of development, however a number of test-peptides have been made in the course of model studies.

A resin bound tetrapeptide PheLeuAlaGly (*109*) was synthesised on an amino methylated resin with a manual synthesiser. This resin has the advantage that direct monitoring of the coupling efficiency of Bnpeoc glycine to the resin can be judged using the Kaiser test.[160] Coupling was accomplished using diphenylphosphinic mixed anhydride in three to four fold excess. Kaiser tests proved that three of the four couplings had gone to completion. However, it was found impossible to obtain a Kaiser-negative result for the final coupling between phenylalanine and leucine. This could be due to the sterically difficult coupling being further inhibited by the general crowding caused by direct attachment of the peptide to the resin with no intermediate

linker or other spacer. The removal of the Bnpeoc group after coupling of Bnpeoc leucine to produce resin (*107*) with 1.2 equivalent DBN and acetic acid in dimethylformamide was monitored by H.P.L.C. From these results the optimum deprotection time with 1.2 equivalents DBN and acetic acid in dimethylformamide using a manual solid phase synthesiser was found to be 10 minutes as discussed in the previous section (Fig.2.25). Hydrolysis of the test-tetrapeptide and amino acid analysis gave the ratio of amino acids as:

Phenylalanine	Leucine	Alanine	Glycine
0.24	0.9	0.97	1

confirming the final coupling was incomplete and the first two were satisfactory.

The Merrifield tetrapeptide leucylalanylglycylvaline[155] was synthesised on a p-alkoxybenzyl alcohol resin with Bnpeoc valine attached either by means of the symmetrical anhydride in the presence of DMAP to give peptide (*110*) or by the acid chloride and pyridine method described in Section 2.5.2 to give peptide (*111*). The Merrifield tetrapeptide has been used many times to test new methodology and it was hoped that these syntheses would provide an opportunity to develop the cycle on an automated synthesiser. For this, the basic Fmoc cycle was used with replacement of the 20% piperidine reagent by a solution of 0.3M DBU and acetic acid in DMA. Couplings were accomplished with DIC[+] and N-hydroxy-benzotriazole with a two-fold excess of all residues, other than glycine in the second synthesis which gave Kaiser-positive results. Both peptides were cleaved from the resin with TFA in dichloromethane and the Z groups were removed

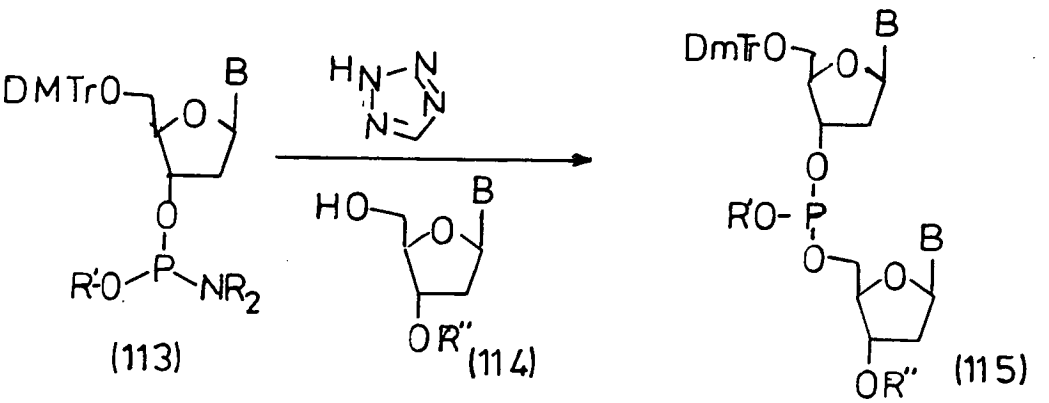
+ DIC = diisopropylcarbodiimide

by catalytic hydrogenation. The resulting deprotected peptides were examined by amino acid analysis. Purification of the peptide obtained by the second synthesis shows removal of much of the impurity following crystallisation (*112*). It is not known as yet what these impurities are but future work should provide more information on this synthesis and the development of a completely reliable cycle for automated synthesis.

2.8 NUCLEOTIDE SYNTHESIS

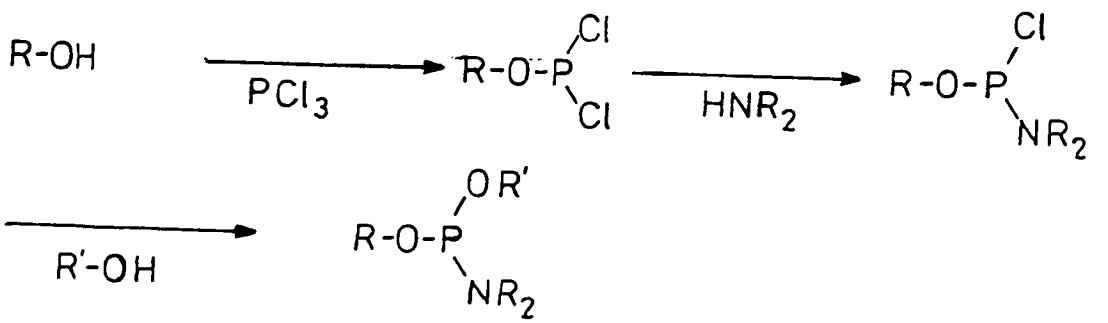
As discussed in Section 1.5 β-eliminating groups have found wide-spread use in nucleotide synthesis. The most successful method for automated oligonucleotide synthesis has been the phosphite triester approach using phosphoramidite intermediates. These intermediates are relatively stable compounds allowing purification by chromatography. Highly reactive intermediates are obtained on activation with tetrazole, thus allowing fast and efficient coupling, see below.

Fig.2.26



From Figure 2.26, R' can be any one of the groups discussed in Sections 1.2 or 1.5. The general method of synthesis for these intermediates is derived from a phosphorodichloridite precursor, by the strategy outlined in Fig.2.27.

Fig.2.27



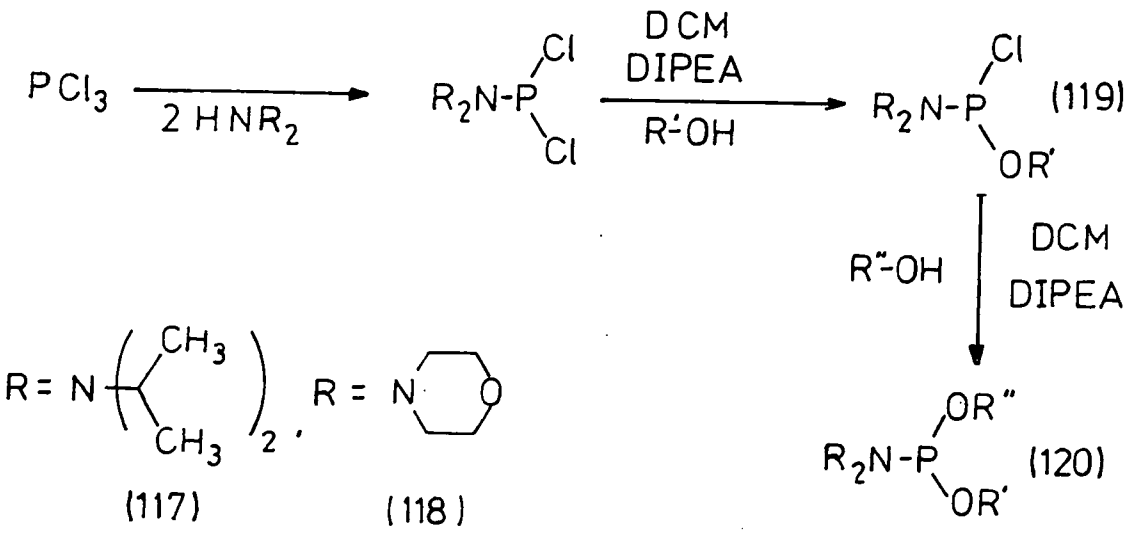
We thought this would be a suitable starting point to investigate the use of the Bnpe-group for protection of the phosphite function illustrated in Fig.2.27. The key intermediate for this route would be the synthesis of 2,2-bis(4-nitrophenyl)ethylphosphoro dichloridite (*116*) the synthesis of which was obtained by the general method described by Claesen, Segers and Tesser[165]. The alcohol (*1*) was added to phosphorus trichloride (PCl_3) in acetonitrite in one portion at room temperature. Should the addition be carried out too slowly, at reduced temperature, or the argon purge be too fast (causing a loss of PCl_3), then di- and tri-substitution of PCl_3 will occur preferentially to (*116*) formation.

General methods for the preparation of the mono-chlorophosphoramidite intermediates (Fig.2.27) require the addition of two equivalents of secondary amine to the phosphorodichloride in diethyl ether. Removal of the amine salt by filtration is generally followed by purification of the product by distillation. Attempts to obtain the mono-chlorophosphoramidite derivative from (*116*) using analogous conditions to those above were unfortunately unsuccessful as (*116*) was found not to be soluble in diethyl ether so other solvents such as THF or dichloromethane had to be used. It was impossible to remove all the amine salt by filtration, due to their finite solubility in the solvents tried. Purification by distillation was not possible as the high boiling point of (*116*) would allow decomposition. It is of considerable importance to remove these salts at this stage as Dörper and Winnacker observed that decomposition of the dichloridite

intermediate can occur in the presence of these salts[166].

A second approach for the synthesis of Bnpe phosphoramidites might prove more successful[163].

Fig.2.28

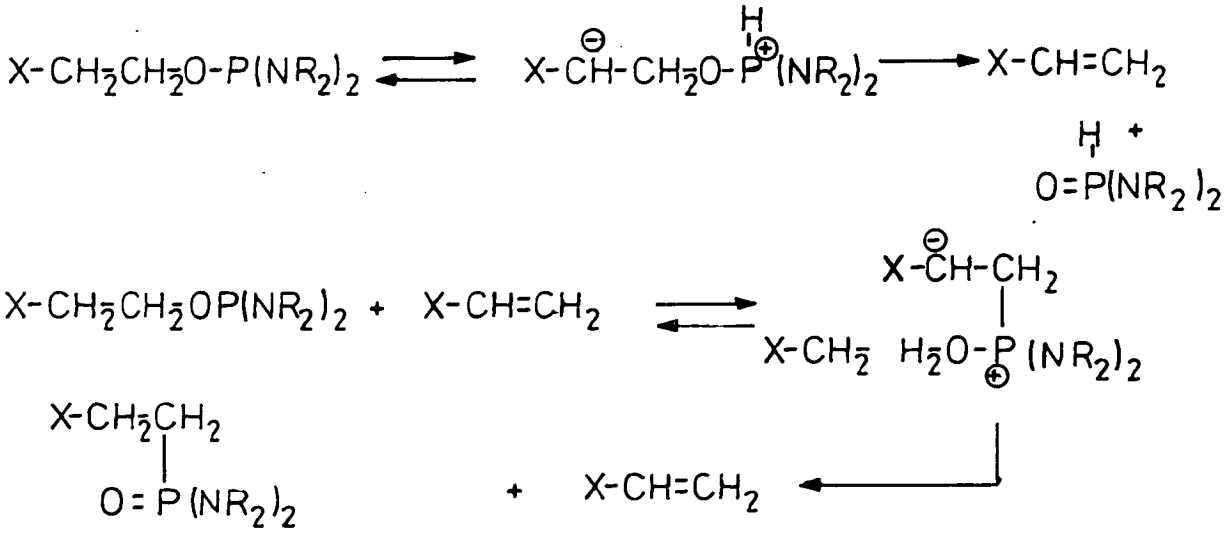


The advantage of the route shown in Fig.2.28 is the ease of synthesis and purification. Dichorodite intermediates were synthesised with diisopropylamine (*117*) and morpholine (*118*) with both compounds obtained pure and crystalline after distillation. Addition of alcohol (*1*) to (*117*) in dichloromethane and diisopropylethylamine was followed by addition of dimethoxytritylthymidine in dichloromethane. However purification on silica gel was thought to cause decomposition of diisopropylamine compounds. Indeed diisopropylphosphoramidites are known not to be completely stable on silica, although the morpholino derivative may possess better general stability[164].

The variable thermal stability of phosphoramidite bearing β-eliminating groups has been studied by Dahl and

co-workers. They found that the stability of these derivatives was increased in line with the bulk of the β-eliminating group. They rationalised this as an inhibitory affect on the nucleophilic nature of the phosphorus by the bulky substituents, preventing proton abstraction by the phosphorus[111].

Fig.2.29



In this study the cyano ethyl group was found to be unstable and the 9-fluoroyl methyl group is known to give reduced coupling efficiency due to steric interference. The Bnpe group might be expected to be intermediate to these two groups and together with the properties discussed in this chapter should provide a useful addition to the range available.

2.8 CONCLUSION

To conclude, this chapter has investigated the synthesis and application of the 2,2-bis(4-nitrophenyl)ethyl-(oxycarbonyl) group. To gauge its overall performance we must return to the stringent criteria set out for the successful development of a novel protecting group in Section 1.6.

The first requirement was that the group should be efficiently cleaved under mild and selective conditions. This has been fulfilled with the development of unique deprotection conditions using stoichiometric amounts of DBN or DBU buffered with an equivalent of acetic acid. Deprotection with these reagents has been shown to be highly effective in either solution or solid phase. These conditions contrast with the current methodology for Fmoc deprotection which uses vast excesses of piperidine in dimethylformamide.

The ease of introduction of a novel protecting group was the second requirement. The initially used protection procedure utilising the chloroformate (*37*) can lead to minor impurities under certain circumstances. However a successful and versatile protection strategy was obtained with the N-succinimidyl carbonate (*42*) derivative which provides high yields of crystalline compounds under standard conditions. The latter point also satisfies the fifth requirement that the reagents for introduction should be readily available and non-toxic.

The prospective protecting group is also required to be complementary to other protecting groups. The Bnpeoc group was found to be stable to acids (Section 2.3.3), tertiary and

primary amines (Section 2.3.2) whilst strong aqueous alkali and secondary amines bring about limited cleavage. Again in contrast to the Fmoc group which was found to be labile to primary amines.

The olefin (*28*) has been shown to be inert under the deprotection conditions developed and can be removed without difficulty from a solid phase synthesis by filtration and by washing the resin with either dimethylacetamide or dichloromethane (Section 2.6) so satisfying the sixth requirement. The final requirement set was that the cost of the protected amino acids be comparative to Boc-protected amino acids. To meet this demand a cheap route to the starting material, alcohol (*1*), must be obtained. The present synthesis, Scheme 2.4, would not allow this requirement to be fulfilled. However the route outlined in Scheme 2.5 is currently under investigation and has provided 2,2-diphenylethanol (*9*) in crystalline yields between 75-80%. It is expected that development of this route will lead to a cheap source of alcohol (*1*) and hence to Bnpeoc amino acids.

The stability of the Bnoe(oc) group has been compared with those of the structurally related protecting groups, Fmoc and p-nitrophenylethyl (NPE). As has been mentioned, the Fmoc group was shown to be more labile than Bnpeoc whilst the NPE group requires relatively stringent conditions to bring about elimination.

These results indicate that the Bnpe(oc) group has intermediate lability with respect to Fmoc and NPE. An added advantage is provided by the convenient storage of Bnpeoc derivatives at room temperature without harm, in contrast

to Fmoc derivatives which require to be stored under refrigeration.

It can be concluded that the Bnpeoc group has satisfied all the requirements set for its successful development. The potential that exists for this group is great and should be fulfilled with its application to solid phase peptide synthesis.

CHAPTER 3

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3.1 NOTES

Melting points were taken in open capillary tubes in an electrically heated Buchi 510 melting point apparatus as well as on a Reichert 7905 hot plate and are used uncorrected. Optical rotations were measured on a Perkin Elmer 141 automatic polarimeter using a 10mm cell, in all cases unless stated otherwise the optical rotation quoted is with dimethylformamide as solvent and C=1. Thin layer chromatography was carried out on plastic sheets coated with silica gel 60GF-254 (Merck) in the following systems: (A) EtOAc: Pet.ether (1:4); (B) EtOAc: Pet.ether (b.p. 40-60) (2:3); (C) $CHCl_3$: MeOH (1:9), (D) $CHCl_3$: MeOH (1:4); (E) $CHCl_3$: MeOH (2:3). Visualisation of the compounds was achieved by a suitable combination of the following methods. U.V. absorption at 254nm, iodine vapour, chlorine starch spray or ninhydrin for peptides with free amino groups. High-performance liquid chromatography (H.P.L.C.) was carried out using a Waters HPLC system comprising 2x6000A pumps, a Waters U6K injector, 680 gradient former, Waters u.v. detector (Model 441), Milton Roy Company computing integrator (Model 308), and hypersil ODS analytical column.

Gradient over 25 minutes, as specified in parentheses, between solvent A (0.05% TFA in water) and solvent B (0.05% TFA in acetonitrile). The flow was 1 ml/min and elution of the samples were recorded at 254nm, retention time given in minutes. Amino acid analysis were carried out on a

LKB 4150 amino acid analyser following sealed tube hydrolysis with constant boiling hydrochloric acid at 110°C for 18 h. Samples for purity analysis were dissolved in 0.2M sodium citrate buffer, pH 3.49 and injected into ion-exchange column (6mm x 240mm) at 75°C. Infra-red spectra were measured on a Perkin Elmer 781 spectrometer. Ultra-violet spectra were measured in distilled methanol on a Pye-Unicam SP-400 spectrometer of Varian Cary 210 spectro-meter. High resolution and low resolution fast atom bombardment (FAB) spectra were measured on a Kratos MSSOTC instrument. Proton n.m.r. spectra were recorded on a Bruker WH360 operating at 360MHz, a Bruker WP200 operating at 200MHz or a Perkin-Elmer R32 operating at 80MHz. Carbon-13 n.m.r. spectra were recorded on a Bruker WP200 operating at 50MHz or a Bruker WP-80-SY at 20.1MHz. All samples were dissolved in deuterated solvents indicated and all chemical shifts were measured relative to TMS. Phosphorus-31 n.m.r. spectra were recorded on a Jeol FX60-Q machine (24.2MHz), chemical shifts were measured relative to 85% phosphoric acid. All solvents were distilled prior to use and the following were dried using the drying agents given in parentheses: N,N-dimethylformamide (DMF) (calcium hydride); ether refers to diethyl ether (sodium); dichloromethane referred to as DCM (calcium hydride); tetrahydrofuran, THF, (benzophenone, sodium); acetonitrile (calcium hydride) refluxed for 18 hours prior to distillation. Distillation of DMF and THF was carried out under reduced (water-pump) pressure. Dicyclo-hexylamine salts of selected protected amino acids were prepared in order to obtain crystalline derivatives.

3.2 PREPARATION OF 2,2-BIS(4-NITROPHENYL)ETHANOL (*1*)

Into anhydrous ammonia (440 ml) was placed freshly cleaned potassium (1 g). This immediately formed a blue colour which was discharged to give a grey suspension on addition of catalytic amount of iron(III) chloride. The remaining potassium (10.3 g, 0.26M) was then added in 1 g amounts. To this suspension was added diphenylmethane (50 g, 0.3M) in dry ether (400 ml) dropwise over 1 hour to give a rust-red precipitate. The liquid ammonia was removed by allowing the mixture to warm to room temperature. The total volume was maintained with addition of ether if necessary. The suspension was heated to reflux to ensure the complete removal of all traces of ammonia.

2,2-Diphenyl ethanol (*9*)

Prepared via 2,2-diphenylacetic acid (*14*)

Method A

Gaseous carbon dioxide (generated from cardice and dried through $CaCl_2$) was passed into a stirred potassium diphenyl methide (0.3M) suspension. The initially rust-red suspension changed to give a grey precipitate which after thirty minutes reaction was washed with water (3x500 ml). The combined brown aqueous phase was acidified to pH 1.5 with concentrated hydrochloric acid. The resulting white precipitate of diphenylacetic acid (*14*) was collected by filtration and dried (56 g, 90%); recrystallised from light

petroleum (b.p. 40-60°C); m.p. 146-147°C (lit. 148°C); t.l.c. - A Rf 0.5.

Esterification of (*14*) (56 g, 0.26M) was achieved by reflux in methanol (250 ml) and H_2SO_4 (30 ml) for 3 hours. The crystalline methyl diphenylacetate (*15*) obtained on cooling the reaction vessel to 0°C was filtered off, additional ester was recovered from the filtrate, total yield 55 g, 92%, m.p. 58-59°C (lit. 60°C), t.l.c. - B Rf 0.6.

Reduction of ester (*15*) (45 g, 0.2M) was accomplished with $LiAlH_4$ (0.6M) in dry ether to give 2,2-diphenylethanol (*9*) (34 g, 0.17M), recrystallised from light petroleum (b.p. 40-60°C), m.p. 55-57°C (lit. 56°C)[125], t.l.c. - B Rf 0.5.

2,2-Diphenylethyl acetate (*7*)

2,2-Diphenylethanol (158 g, 0.8M) in pyridine (250 ml) and acetic anhydride (90 g, 0.88M) was stirred for five hours at room temperature. After removal of the solvent (co-operated with toluene) 2,2-diphenylethyl acetate (*7*) was obtained as a white solid, 167 g, 87%; recrystallised from diethylether; m.p. 54-55°C, t.l.c. - A Rf 0.5.

2,2-Bis(4-nitrophenyl)ethyl acetate (*8*)

2,2-Diphenylethyl acetate (*7*), (96 g, 0.4 mol) was added in small portions as a solid to a vigorously stirred mixture of conc. H_2SO_4/conc. HNO_3 (122 ml:122 ml) at -10°C. Addition over 1 hour allowed the temperature of the reaction

to be maintained below 0°C. The reaction was stirred for thirty minutes at room temperature before quenching into ice/water to produce a thick yellow gum. The supernatant liquid was decanted to allow the gum to be washed with fresh amounts of water whilst sodium hydroxide was added to obtain a neutral pH. This was repeated with further amount of water until the washings were of neutral pH without addition of alkali. The neutralised gum was crystallised from diethyl ether to give 2,2-Bis(4-nitrophenyl)ethyl acetate (*8*), 50 g, further material was obtained from the filtrate, total yield 60%, recrystallised from chloroform/ light petroleum (b.p. 40-60°C); m.p. 107-108°C; (Found: C, 58.5; H, 4.33; N, 8.45; $C_{16}H_{14}N_2O_6$ requires: C, 58.2; H, 4.2; H, 8.5); t.l.c. - A Rf 0.2; λ_{max} 27.4 (ε19500); ν_{max} (CH_2Cl_2) 1749 (CO, acetate), 1605, 1595, (C-C, aromatic), 1520, 1350 (NO_2), 1240, 1220, 1110, 1040 (C-O) and 860 cm^{-1} (p-disubst. benzene); δ_H ($CDCl_3$, 200MHz) 8.09 (4H, d, J_{AB} 8.7Hz), 7.47 (4H, d, J_{AB} 8.7Hz) (aromatic 2xAB protons) 4.63 (3H, m_{AB_2} CH CH_2), 1.9 (3H, s, CH_3); δ_c ($CDCl_3$, 50MHz) 170.0 (CO, acetate), 146.8 (quaternary aromatic C's x2), 128.9, 123.6 (aromatic CH's), 64.9 (CH_2), 49.1 (CH), 20.1 (CH_3); m/z (FAB), HRMS; Found: 331.09300, $C_{16}H_{15}N_2O_6$ requires 331.09300 (∴ = <0 ppm).

2,2-Bis(4-nitrophenyl)ethanol (*1*)

(*8*) (30 g, 0.09M) was dissolved in a mixture of methanol (285 ml) and conc. HCl (aq) (94 ml) under reflux. After three hours the reaction was quenched into ice/water yielding a yellow solid. The solid was dissolved in

ethyl acetate and combined with the ethyl acetate extraction (3 x 50 ml) of the aqueous phase. The organic phase was dried over $MgSO_4$ and the solvent removed *in vacuo* to produce 2,2-Bis(4-nitrophenyl)ethanol (*1*) as a yellow solid, crystallised from chloroform/diethyl ether (24 g, 94%); recrystallisation from chloroform gave a white solid with a pale green hue; m.p. 109-110°C, (Found: C, 58.0; H, 4.2; N, 9.7; $C_{14}H_{12}N_2O_5$ requires: C, 58.3; H, 4.2; N, 9.7); t.l.c. - B Rf 0.20, ν_{max} (CH_2Cl_2) 3605 (OH), 1605, 1595, (C-C, aromatic), 1520, 1350 (NO_2), 1430, 1110, 1090, 1060 (C-O) and 860 cm^{-1}, λ_{max} 274 (ε 21000); δ_H [$(CD_3)_2CO$, 200 MHz] 8.19 (4H, d, J_{AB} 8.9Hz), 7.65 (4H, d, J_{AB} 8.8 Hz), 4.51 (1H, m_{AB_2}, benzyl H), 4.27 (2H, d_{B_2A}, CH_2), 2.89 (1H, broad alcohol peak); δ_c [$(CD_3)_2CO$, 90.5 MHz] 149.54, 147.04 (quaternary aromatic C), 129.90, 123.64 (aromatic CH's), 64.52 (CH_2), 53.29 (CH); m/z (FAB) H.R.M.S. Found: 289.0834; $C_{14}H_{13}N_2O_5$ requires 289.0834 (.˙. = <1 ppm).

3.3 PREPARATION OF 2,2-BIS(4-NITROPHENYL)ETHYL DERIVATIVES

2,2-Bis(4-nitrophenyl)ethyl chloride (*27*)

2,2-Bis(4-nitrophenyl)ethanol (*1*) (1.35 g, 4.7 mmol) and triphenyl phosphine (1.3 g, 4.7 mmol) were dissolved in tetrachloromethane (20 ml) with stirring. After three hours reflux the solvent was removed under reduced pressure. Chromatography of the residue (ethyl acetate/light petroleum (b.p. 40-60°C); 1:4) on silica gel afforded 2,2-Bis(4-nitrophenyl)ethyl chloride (*27*) (1. g, 50%); recrystallised from chloroform/diethylether; m.p. 131.5-132°C; (Found: C, 55.0; H, 3.52; N, 9.12; $C_{14}H_{11}N_2O_4Cl$ requires: C, 54.8;

H, 3.60; N, 9.10); t.l.c. - B, Rf 0.6; ν_{max} (CH_2Cl_2) 1605, 1595 (C-C, aromatic), 1520 and 1350 (NO_2) cm^{-1}. λ_{max} 274 (ε 12750); δ_H [$(CD_3)_2CO$, 80 MHz] 8.24 (4H, d, J_{AB} 8.8 Hz), 7.75 (4H, d, J_{AB} 8.8 Hz), 4.88 (1H, m_{AB_2} CH), 4.41 (2H, m_{AB_2}, CH_2); δ_c [$(CD_3)_2CO$, 50 MHz] 147.2, 146.5 (quaternary aromatic C's), 128.6, 122.9 (aromatic CH's), 51.9 (CH), 44.8 (CH_2); m/z (FAB); Found: 309.04559, $C_{14}H_{11}H_2O_4Cl$ requires: 309.04560 (.˙. = <1 ppm).

2,2-Bis(4-nitrophenyl)ethyl bromide (*26*)

To a stirred solution of 2,2-Bis(4-nitrophenyl)ethanol (*1*) (0.16 g, 0.54 mmol) in dichloromethane (10 ml) was added carbon tetrabromide (0.362 g, 1.08 mmol) and triphenylphosphine (0.29 g, 1.08 mmol). The initially clear solution turned yellow immediately and t.l.c. indicated that the reaction was complete within five minutes. The solvent was removed *in vacuo* to produce a gum which after chromatography on silica gel (ethyl acetate/light petroleum (b.p. 40-60°C) (1:4) provided 2,2-Bis(4-nitrophenyl)ethyl bromide (0.14 g, 75%); recrystallised from dichloromethane, m.p. 142-144°C; (Found: C, 47.96; H, 3.13; N, 7.96; $C_{14}H_{11}N_2O_4Br$ requires: C, 47.86; H, 3.13; N, 7.98); t.l.c. - B, Rf 0.6; ν_{max} (CH_2Cl_2) 1605, 1595, 1520, 1350 (NO_2) and 860 cm^{-1}; λ_{max} 274 (ε 20600) δ_H ($CDCl_3$, 80 MHz) 8.20 (4H, d, J_{AB} 8.8 Hz), 7.41 (4H, d, J_{AB} 8.7 Hz), 4.62 (1H, t, J_{AB_2} 7.6 Hz), 3.95 (2H, d J_{AB_2} 7.5 Hz); δ_c ($CDCl_3$, 50 MHz) 147.3 (2. quaternary aromatic C's), 128.8, 124.0 (aromatic CH's), 52.8 (CH), 32.9 (CH_2), m/z (FAB); Found: 350.99807, $C_{14}H_{12}H_2O_4Br$ requires: 350.9909 (.˙. = <1 ppm).

1,1-Bis(4-nitrophenyl)ethene (*28*)

This compound was prepared by elimination of 2,2-Bis(4-nitrophenyl)ethyl acetate (*8*) (2.1 g, 6.24 mmol) in chloroform (25 ml) with DBN (2 equivs.). On addition of DBN the initially clear solution turned dark pink with a white precipitate . After 15 minutes the reaction was washed with 2M HCl (2 x 15 ml), 0.1M NaOH (2 x 15 ml), water, brine and finally dried over $MgSO_4$. The solvent was removed *in vacuo* to produce white/yellow crystals (1.6 g, 95%); m.p. 174-176°C (lit. 175-176°C)[115] ν_{max} (CH_2Cl_2) 1605, 1595 (C-C, aromatic), 1420, 1520, 1350 (NO_2); t.l.c. - B, Rf 0.6 λ_{max} 300 (ε 16000).

2,2-Bis(4-nitrophenyl)ethyl chloroformate (*37*)

Method A

(*1*) (14.4 g, 50 mmol) was dissolved in abs.toluene (200 ml) at 60°C. To this solution, maintained at 40°C, was added phosgene (64.4 ml, 1.5 eq., 12½% soln w/w toluene) followed by N-methylmorpholine (6 ml). After 30 minutes the precipitated N-methylmorpholine hydrochloride was removed *via* filtration through a grade 3 sintered funnel to leave a clear green solution. The solvent was removed *in vacuo* to produce a green oil which was crystallised from diethyl ether/chloroform to give 2,2-Bis(4-nitrophenyl)-ethyl chloroformate (*37*) as a white solid (17.5 g, 100%); recrystallised from chloroform/diethyl ether, m.p. 97-98°C; (Found: C, 51.4; H, 3.11; N, 8.01; $C_{15}H_{11}N_2O_6Cl$ requires:

C, 51.4; H, 3.14; N, 7.99), ν_{max} (CH_2Cl_2) 1770 (CO) 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1160, 1145, 860 and 820 cm^{-1}; λ_{max} 274 (ε 20400); δ_H ($CDCl_3$, 200 MHz) 8.19 (4H, d, J_{AB} 8.7 Hz), 7.42 (4H, d, J_{AB} 8.7 Hz), 4.91 (2H, d, J_{AB_2} 7.1 Hz, CH_2), 4.70 (1H, t, J_{AB_2} 7.0 Hz, CH); δ_C ($CDCl_3$, 50 MHz) 150.4 (CO), 147.4, 145.4 (quaternary aromatic C's), 129.0, 124.1 (aromatic C's), 71.6 (CH_2), 48.9 (CH); m/z (FAB); Found: 351.03839, $C_{15}H_{12}N_2O_6Cl$ requires: 351.03838 (∴ = <1 ppm).

Method B

(*1*) (9.9g, 35 mmol) was dissolved in dichloromethane (150 ml) and cooled to 0°C. To this solution was added phosgene (28.1 ml, 51.7 mmol., 1 eq.) and N-methylmorpholine (3.8 ml, 34.5 mmol, 1 eq.) dropwise over twenty minutes. After stirring at room temperature for thirty minutes the solid N-methylmorpholine hydrochloride was filtered off to leave a cloudy green solution. The solvent was removed *in vacuo* to produce a green gum/crystalline residue which was redissolved in dioxan.

(i) This solution could be used directly for the synthesis of 2,2-Bis(4-nitrophenyl)ethyl N-succinimidyl carbonate (*42*) or allowed the isolation of the chloroformate (see (ii)).

(ii) Any remaining N-methyl morpholine salt was removed by filtration of this solution to produce a clear green liquid. The solvent was removed *in vacuo* and the residue crystallised as for Method A (10.4 g, 86%).

2,2-Bis(4-nitrophenyl)ethyl N-succinimidyl carbonate (*42*)

The chloroformate (*37*) (55.1 g, 0.157M) in 1,4-dioxan (600 ml), produced by Method B, together with N-hydroxysuccinimide (19.9 g, 0.173M) dissolved in 1,4-dioxan (300 ml) were cooled to 0°C. N-Methyl morpholine (17.4 ml, 0.156M) was added at a rate to keep the temperature between 10-15°C. After stirring for 2½ hours at room temperature the precipitated N-methylmorpholine hydrochloride was filtered off to leave a clear green liquid. The solvent was removed *in vacuo* to produce a white solid; crystallised from ethyl acetate to give pure 2,2-Bis(4-nitrophenyl)ethyl N-succinimidyl carbonate (*42*) (50.8 g, 75% based on 2,2-Bis(4-nitrophenyl)ethanol (*1*)); recrystallised from acetone/light petroleum (b.p. 40-60°C), m.p. 173-174°C (Found: C, 52.8; H, 3.5; N, 9.7; $C_{19}H_{15}N_3O_9$ requires: C, 53.1; H, 3.5; N, 9.8); t.l.c. - C, Rf 0.5; ν_{max} (CH_2Cl_2) 1810, 1795, 1750 (CO's), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1220, 1205, 1120 and 875 cm^{-1}; λ_{max} 274 (ε 15431), δ_H [$(CD_3)_2CO$, 200 MHz] 8.3 (4H, d, J_{AB} 8.8 Hz), 7.7 (4H, d, J_{AB} 8.8 Hz), 5.2 (3H, m_{AB_2}, CH, CH_2, Bnpeoc) 2.8 (4H, s, CH_2x2); δ_C [$(CD_3)_2$ CO, 50 MHz] 168.2 (CO, succinimide), 150.7 (CO, carbonate), 146.7, 145.9 (quaternary aromatic C's), 128.8, 123.1 (aromatic CH's), 70.5 (CH_2) 48.3 (CH), 24.5 (CH_2's, succinimide); m/z (FAB) HRMS: Found: 430.08861; $C_{19}H_{16}N_3O_9$ requires 430.0886 (∴ <1 ppm).

2,2-Bis(4-nitrophenyl)ethyl cinnamate (*29*)

To a cooled solution of (*1*) (1.81 g, 5.3 mmol) and

cinnamic acid (*30*) (0.86 g, 5.83 mmol) in dry dichloromethane (20 ml) was added dicyclohexylcarbodiimide (1.31 g, 6.4 mmol) in dichloromethane (5 ml) and a catalytic amount of 4-dimethylaminopyridine over twenty minutes. After stirring for ninety minutes the reaction was cooled to -20°C and the precipitated dicyclohexylurea filtered off. The filtrate was reduced *in vacuo* to produce a residue which was dissolved in ethyl acetate and cooled to -20°C; any precipitate formed was removed by filtration to leave a clear green solution. The solvent was removed under reduced pressure to produce a brown gum which was crystallised from diethyl ether to give pure 2,2-Bis(4-nitrophenyl)ethyl cinnamate (*29*) (1.6 g, 70%); recrystallised from dichloromethane diethyl ether, m.p. 97-98°C; (Found: C, 66.2; H, 4.29; N, 6.70; $C_{23}H_{18}N_2O_6$ requires: C, 66.0; H, 4.3; N, 6.70); t.l.c. - A, Rf 0.3; B, Rf 0.5; ν_{max} (CH_2Cl_2) 1715 (CO), 1630 (C=C), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2) and 1160 cm^{-1}; λ_{max} 277 (ε 36800); δ_H ($CDCl_3$, 80 MHz) 8.1 (4H, d, J_{AB} 8.5 Hz), 7.7 - 7.4 (9H, m, aromatic CH's) 6.4, 6.2 (2H, m, olefin), 4.7 (3H, m, CH, CH_2); δ_c ($CDCl_3$, 50 MHz) 166.1 (CO), 147.1, 146.8, 145.7 (quaternary aromatic C's), 133.8 (aromatic CH), 130.4 (olefin CH), 129.0, 128.7, 127.9, 123.8 (aromatic CH's) 116.8 (olefin CH), 65.1 (CH_2), 49.4 (CH),; m/z (FAB) HRMS, Found: 419.1243: $C_{23}H_{18}N_2O_6$ requires 419.1243 (.˙.<1 ppm).

4-Nitrophenylethyl cinnamate (*31*) was prepared from 4-nitrophenyl ethanol (*32*) (1.0 g, 6.05 mmol) and cinnamic

acid (0.99 g, 6.7 mmol) as described for the preparation of 2,2-Bis(4-nitrophenyl)ethyl cinnamate (*29*). Pure (*31*) was obtained as white crystalline solid from dichloromethane/ diethylether (1.5 g, 85%), m.p. 101-103°C; t.l.c. - B, Rf 0.6, ν_{max} (CH_2Cl_2) 1710 (CO), 16.30 (C=O), 1600 (C-C aromatic), 1520, 1350 (NO_2) and 1170 cm^{-1} (C-O); δ_H ($CDCl_3$, 80 MHz) 8.14 (2H, d, J_{AB} 8.7 Hz) 7.74 7.74-7.3 (7 H, m, aromatic CH's), 6.46, 6.26 (olefin, CH's), 4.44 (2H, $J_{A_2B_2}$ 6.7 Hz), 3.10 (2H, t, $J_{A_2B_2}$ 6.7 Hz); δ_C ($CDCl_3$, 50 MHz) 166.5 (CO), 146.8, 145.6, 145.2 (quaternary C's), 134.1, 130.3, 129.6, 128.3, 128.0, 123.6, 117.4 (olefinic and aromatic CH's), 63.7 (CH_2), 34.7 (CH); m/z (FAB) L.R.M.S. 298, 289, 154, 136, 131, 120, 108, 91; H.R.M.S. Found: 298.10793, $C_{17}H_{16}NO_4$ requires: 298.10792 (.˚.=<1 ppm).

3.4 PREPARATION OF N-[2,2-BIS(4-NITROPHENYL)ETHYLOXY-CARBONYL AMINOACIDS

As a representative example of Route 1, preparation of N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-2-alanine (41) Bnpeoc Ala-OH

Route 1

Alanine (0.78 g, 8.9 mmol) was dissolved in aqueous 10% Na_2CO_3 solution (5.4 ml, 5.2 mmol) and cooled to 0°C. To this was added Bnpeoc ONSu (*42*) (3 g, 7.1 mmol) in dimethylformamide (10 ml). The lumpy white suspension formed was stirred for 15 minutes at room temperature before the addition of 5 volumes of water. The aqueous mixture was extracted with ether (50 ml x 2) and ethyl acetate (50 ml x 2) to produce a clear, green solution. The cooled aqueous phase was acidified with conc.HCl to pH 1.5 and extracted with ethyl acetate (50 ml x 4). The combined organic phase was washed with water, brine and dried over $MgSO_4$. The solvent was removed *in vacuo* to produce a green gum crystallised from diethyl ether/chloroform to give pure N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]alanine (3 g, 89%), recrystallised from diethyl ether/chloroform; m.p. 148-149.5°C; (Found: C, 53.51; H, 4.20; N, 10.41; $C_{18}H_{12}N_3O_8$ requires: C, 53.60; H, 4.18; N, 10.40); $[\alpha]_D^{27}$+0.7; t.l.c. - C, Rf 0.3; ν_{max} (CH_2Cl_2) 3425 (NH), 3040 (CH, aromatic), 2980 (CH, alkyl), 1730 (CO, urethane, acid), 1605, 1595, (C-C, aromatic), 1520, 1350 (NO_2), 1420, 1280, 1080, 890 and 860 cm^{-1}; λ_{max} 274 (ε 32400); δ_H [$(CD_3)_2CO$, 200 MHz] 8.22 (4H, d, J_{AB} 8.8 Hz)

7.70 (4H, d, J_{AB} 8.8 Hz), 6.6 (1H, m, NH), 4.75 (3H, d, J 7.3 Hz, CH_3); δ_c [$(CD_3)_2CO$, 50 MHz], 17.3 (CO, acid), 155.6 (CO, urethane), 148.2, 146.7 (quaternary aromatic C's), 129.7, 123.8 (aromatic CH's), 65.2 (CH_2, Bnpeoc), 49.3 (CH, Bnpeoc), 49.15 (αCH), 17.0 (CH_3, ala); m/z (FAB) HRMS, Found: 404.10939, $C_{18}H_{18}N_3O_8$ requires: 404.1094 (.˙.<1 ppm).

Modifications to Route 1

It has been found necessary to increase the reaction time for some amino acids from fifteen minutes to several hours depending on the scale.

In the case of amino acids with t-butyl ester (or other acid sensitive protecting group) side chain protection, saturated citric acid solution was used instead of concentrated HCl in the work-up.

For the preparation of Bnpeoc-Met-OH (*62*) and Bnpeoc Trp-OH (*63*) reaction and work-up was performed under nitrogen.

Route 2

For amino acids found difficult to dissolve in 10% sodium carbonate 1M sodium hydroxide was used in its place (see Bnpeoc Tyr-OH (*67*)).

Route 3

Protection of sterically hindered amines or amines with reduced nucleophilicity was achieved using 2,2-Bis(4-nitrophenyl)ethyl chloroformate (*37*) [see Bnpeoc Aib-OH (*61*)] with a Na_2CO_3 solution diluted with DMF (1:1) work-up

of these reactions was as for Route 1, other than for acid sensitive molecules in which case the modified Route 1 for acid labile groups was used.

3.4.1 Preparation of dicyclohexylammonium salts

As a representative example, preparation of N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-tryptophan dicyclohexylammonium salt

Dicyclohexylamine (0.27 ml, 1.36 mmol) was added to a cooled solution of Bnpeoc Trp-OH (0.64 g, 1.1 mmol) in a minimum volume of dichloromethane (3 ml) and stirred for 5 minutes at 0°C and then 5 minutes at room temperature. To this was added sufficient diethylether until a crystalline product was obtained (0.51 g, 60%).

Regeneration of the free acid was achieved for Bnpeoc-Ile (*49*) by chromatography on silica ($CHCl_3$: MeOH, 99:1, chromatron), to give pure (*49*) in quantitative yield.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl] amino isobutyric acid (*61*), Bnpeoc Aib-OH was prepared by Route 3 to give a white foam (0.6 g, 50%); crystallised from diethylether/chloroform; recrystallised from ethyl acetate/light petroleum (b.p. 40-60°C), m.p. 166-169°C; (Found: C, 54.7; H, 4.59; N, 10.1; $C_{19}H_{19}N_3O_8$ requires: C, 55.0; H, 4.77; N, 9.90); t.l.c. - D, Rf 0.5; ν_{max} (CH_2Cl_2) 3420 (NH), 2980 (CH, alkyl), 1720 (CO, urethane, acid), 1605, 1595

(C-C, aromatic), 1520, 1350 (NO_2), 1415, 1095, 1110, 1120 (C-O) and 860 cm^{-1}; λ_{max} 275 (ε 19850); δ_H [$(CD_3)_2CO$, 200 MHz] 8.22 (4H, d, J_{AB} 8.8 Hz), 7.69 (4H, d, J_{AB} 8.8 Hz), 4.77 (3H, m_{AB_2}, $CHCH_2$, Bnpeoc], 1.45 (6H, s, CH_3 x 2); δ_c [$(CD_3)_2CO$, 50 MHz] 174.2 (CO, acid), 153.9 (CO, acid), 147.3, 146.5 (quaternary aromatic C's), 128.9, 122.8 (aromatic CH's), 64.5 (CH_2, Bnpeoc), 54.9 (αCH), 49.1 (CH, Bnpeoc), 23.9 (CH_3 x 2); m/z (FAB) HRMS, Found: 418.12499; $C_{19}H_{20}N_3O_8$ requires 418.1250 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-asparagine (45)

Bnpeoc Asn-OH was prepared by Route 1.

Yield, 75%, recrystallised from acetone/light petroleum (b.p. 40-60°C), (Found: C, 50.89; H, 4.05; N, 12.60; $C_{19}H_{18}N_4O_8$ requires C, 51.1; H, 4.04, N, 12.46); m.p. 177-178°C, $[\alpha]_D^{27}$+0.5, t.l.c. - E, Rf 0.2; ν_{max} (Bromoform mull) 3430, 3140 (b.s.) (amide NH_2), 2970-2550 (H-bonding), 1750 (CO, urethane), 1705 (CO, acid), 1675 (amide), 1520, 1350, (NO_2), 1270, 1240, 1205 (C-O), 1080, 860 and 840 cm^{-1}; λ_{max} 274 (ε 23200), 219, 206; δ_H [$(CD_3)_2CO$, 200 MHz] 8.14 (4H, m, $4CH_A$), 7.70 (4H, m, 4 x CH_B), 7.30 (2H, m, NH_2), 6.90 (1H, m, NH), 4.70 (3H, m, $CHCH_2$, Bnpeoc); m/z (FAB), HRMS, Found: 447.11522, $C_{19}H_{19}N_4O_9$ requires 447.1152 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-6-aminopenicillanic acid (60), Bnpeoc APA-OH was prepared by the modified Route 3.

Yield 82%, white powder from diethyl ether/light petroleum

(b.p. 40-60°C), ν_{max} (CH_2Cl_2) 3420 (NH), 3030 (CH, aryl), 2980 (CH, alkyl), 1790 (CO, β-lactam), 1730 (CO, urethane, acid), 1605, 1595 (C-C, aromatic), 1520 and 1350 cm^{-1} (NO_2); λ_{max} 274 (ε21000); δ_H [$(CD_3)_2CO$, 200 MHz) 8.22 (4H, d, J_{AB} 8.6 Hz) 7.70 (4H, d, J_{AB} 8.6 Hz), 5.5 (2H, m, β-lactam CH's), 4.8 (3H, m, $CHCH_2$-Bnpeoc), 4.8 (1H, C_3H), 1.5 (6H, m, 2 x CH_3), δ_c [$(CD_3)_2CO$, 50 MHz], 167.4 (CO, β-lactam), 154.0 (CO, urethane), 147.0, 146.4 (quaternary aromatic C's), 128.8 122.9 (aromatic CH's), 69.5 (β-lactam CH), 67.1 (quaternary C), 65.4 (CH_2, Bnpeoc), 63.2 (β-lactam CH), 58.1 (αCH), CH_3's hidden by $(CD_3)_2CO$ signal; m/z (FAB), Found: 531.11853, $C_{23}H_{23}N_4O_9S$ requires 531.11856 (.˙.=<1 ppm). $[\alpha]_D^{27}$ +119.0.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl] -7-amino cephalosporanic acid (59), Bnpeoc ACA-OH was prepared by modified Route 3, from 7-aminocephalosporonic acid (1.2 g, 4.4 mmol) and Bnpeoc-Cl (1.33 g, 3.8 mmol) to give a brown powder (1.6 g, 72%) from light petroleum (b.p. 40-60°C); ν_{max} (CH_2Cl_2) 3420 (NH), 3020 (CH, aryl), 2980 (CH, alkyl), 1790 (CO, β-lactam), 1740 (CO, acid, urethane), 1670, 1640 (C-C, olefin), 1605, 1595 (C-C, aryl), 1520, 1350 (NO_2), 1225, 1110 and 1050 cm^{-1} (C-O); δ_H ($CDCl_3$, 80 MHz) 8.2 (4H, m), 7.4 (4H, m), 5.8 (2H, β-lactam CH's), 5.0 (2H, m, H_2C-5), 4.65 (3H, m, $CHCH_2$-Bnpeoc), 3.45 (2H, m, CH_2OAc), 2.0 (3H, s, OCH_3), δ_c [$(CD_3)_2CO$, 50 MHz] 169.1 (CO, acetate), 163.3 (CO, β-lactam), 161.3 (CO, acid), 154.6 (CO, urethane), 147.0, 146.6 (quaternary aromatic), 128.9, 122.9 (aromatic CH's), 125.2 (olefin C's), 65.6 (CH_2, Bnpeoc), 62.0 (CH_2-S), 60.7, 57.0 (β-lactam CH's), 48.9

(CH, Bnpeoc), 25.0 (CH_2OAc), 18.9 ($O-CH_3$); m/z (FAB) HRMS; Found 587.10836; $C_{25}H_{23}N_4O_{11}S$ requires 587.10839 (.'.<=1 ppm), $[\alpha]_D^{27}$ -46.9.

Nα-2,2-Bis(4-nitrophenyl)ethoxycarbonyl nitro arginine (46), Bnpeoc Arg(NO_2)OH (46)

Prepared by Route 1.

Amorphous white powder from light petroleum (b.p. 40-60°C) $[\alpha]_D^{27}$+0.3 δ_H($CDCl_3$, 200 MHz) 8.22 (4H, d, J_{AB} 8.8 Hz), 7.70 (4H, d, J_{AB} 8.8 Hz), 4.77 (3H, m, $CHCH_2$, Bnpeoc), 4.20 (1H, m, α CH), 3.36 (2H, m, δCH_2), 1.75 (4H, m, $\beta,\gamma CH_2$); δ_C [$(CD_3)_2CO$, 50 MHz] 1720 (CO, acid), 159.2 (quaternary guanadino C), 155.1 (CO, urethane), 147.3, 146.4 (quaternary aromatic C's), 128.9, 122.9 (aromatic CH's), 64.9 (CH_2, Bnpeoc), 58.8 (αCH), 48.9 (CH, Bnpeoc), 39.7 (δCH_2), 27.7 (βCH_2), 24.5 (γCH_2); m/z (FAB) HRMS, Found: 534.15843; $C_{21}H_{24}N_7O_{10}$ requires 534.1584 (.'.=<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-glutamine (47) Bnpeoc Gln-OH prepared by Route 1

Yield 68%; recrystallised from acetone/light petroleum (b.p. 40-60°C); (Found: C, 52.4; H, 4.35; N, 12.0; $C_{20}H_{20}N_4O_9$ requires: C, 52.17; H, 4.35; N, 12.17); ν_{max} (bromoform mull), 3420, 3340, 1750 (CO, methane), 1705, 1675 (CO, acid, amide), 1590-1555, 1520, 1350 (NO_2) λ_{max} 274 (ε20000), 215 (ε22000); δ_H [$(CD_3)_2SO$, 200 MHz] 8.19 (4H, d, J_{AB} 8.4 Hz), 7.68 (4H, d, J_{AB} 8.4 Hz), 4.77 (3H, m_{AB_2} $CHCH_2$Bnpeoc), 3.91 (1H, m, α CH) 2.08 (3H, m_{AB},

γCH_2, Gln), 1.80 (2H, m_{AB}, βCH_2, Gln); m/z (FAB) HRMS, Found 461.13083; $C_{20}H_{21}N_4O_9$ requires 461.1308.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-glutamic acid (γ^t-Butyl ester) (*57*)

Bnpeoc Glu (^{t}Bu) OH was prepared by Route 2. The crude product was purified on silica gel (chromatron, $CHCl_3$) to give (*57*) as a foam (50%), amorphous powder from light petroleum (b.p. 40-60 °C), t.l.c. - C, Rf 0.4; ν_{max} (CH_2Cl_2) 3920 (NH), 2980 (alkyl CH), 1720 (CO, urethane, acid), 1605, 1595 (C-C, aromatic), 1520, 1350 (NH_2), 1420, 1280, 1240, 1155, 1110 (C-O), 880 and 860 cm^{-1}; δ_H ($CDCl_3$, 200 MHz) 8.16 (4H, d, J_{AB} 8.8 Hz), 7.39 (4H, d, J_{AB} 8.8 Hz), 5.52 (1H, d, J_{AB} 8.8 Hz), 4.70 (3H, m, $CHCH_2$, Bnpeoc), 4.29 (1H, m, αCH), 2.28 (2H, m, γCH_2), 2.1 (2H, m, βCH_2), 1.40 (9H, s, CH_3 x 3); δ_C ($CDCl_3$, 50 MHz) 175.3 (CO, acid) 172.1 (CO, ester), 155.6 (CO, urethane), 1470, 1468 (quaternary aromatic C's), 129.0, 123.8 (aromatic CH's), 81.0 (quaternary c-^{t}Butyl), 65.9 (CH_2, Bnpeoc), 53.3 (αCH), 49.5 (CH, Bnpeoc), 31.3 (δCH_2), 27.7 (CH_3 x 3), 26.7 (βCH_2).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-glutamic (γ^tButyl ester) dicyclohexylammonium salt (*58*)

Obtained *via* the general procedure of 3.4.1 with (*57*) (0.45 g, 0.92 mmol) in dichloromethane (5 ml); crystallised from diethylether/dichloromethane, m.p. 124.5-126; (Found: C, 62.2; H, 7.3; N, 8.1; $C_{36}H_{48}N_4O_{10}$ requires

C, 62.1; H, 6.9; N, 8.0); $[\alpha]_D^{27}$+5.2, t.l.c. - C, Rf as for (57); λ_{max} 274 (ε20200), δ_H ($CDCl_3$, 200 MHz) 8.14 (4H, d, J_{AB} 8.7 Hz), 7.37 (4H, d, J_{AB} 8.7 Hz), 4.6 (3H, m_{AB_2}, $CHCH_2$ Bnpeoc), 3.9 (1H, m, αCH), 2.9 (2H, m, 1CH, DCHA), 1.8 (16H, m, β,γ CH_2, Glu, 12H DCHA), 1.36 (9H, s, CH_3 x 3), 1.25 (8H, m, CH_2 x 4, DCHA); δ_c ($CDCl_3$, 50 MHz) 175.0 (CO, acid), 172.4 (CO, ester), 155.0 (CO, urethane), 147.2 (2 x quaternary C's), 129.1, 123.8 (aromatic CH's), 79.9 (quaternary ^{t}Butyl-C), 65.5 (CH_2, Bnpeoc), 55.3 (1CH,DCHA), 52.8 (αCH, Glu), 49.9 (CH, Bnpeoc), 29.2 (CH_2, DCHA), 28.7 (CH_3 x 3), 25.1 (CH_2, DCHA), 24.6 (CH_2, DCHA); m/z (FAB), HRMS; Found 699.36050; $C_{36}H_{51}N_4O_{10}$ requires 699.36049.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]glycine (48)

Bnpeoc Gly-OH Prepared by Route 1.

Yield 90%, recrystallised from ethyl acetate/light petroleum (b.p. 40-60°C); m.p. 156-158°C, (Found: C, 52.4; H, 3.88; N, 10.80; $C_{17}H_{15}N_3O_8$ requires C, 52.8; H, 3.86; N, 10.80), t.l.c. - D, Rf 0.23; ν_{max} (CH_2Cl_2) 3470 (NH), 1730 (CO, urethane, acid), 1605 (aromatic C-C), 1520 and 1350 cm^{-1} (NO_2); λ_{max} 275 (ε19500), δ_H [$(CD_3)_2CO$, 200 MHz] 8.22 (4H, d, J_{AB} 8.7 Hz), 7.70 (4H, d, J_{AB} 8.7 Hz), 6.63 (1H, m, NH), 4.77 (3H, m, CH,CH_2, Bnpeoc), 3.86 (2H, m_{AB}, CH_2,Gly), δ_c [$(CD_3)_2SO$, 200 MHz, DEPT] 129.5, 123.7 (aromatic CH's), 65.1 (CH_2, Bnpeoc), 48.9 (CH, Bnpeoc), 42.0 (CH_2,Gly); m/z (FAB), LRMS, 390, 361, 345, 299, 283, 270, 254, 241, 225, 179, 150, 137, 121, 105; HRMS, Found: 390.09372; $C_{17}H_{16}N_3O_8$ requires 390.0937 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-isoleucine (*49*), Bnpeoc Ile-OH prepared by Route 1

Yield 73%, amorphous white powder from light petroleum (b.p. 40-60°C), (Found: C, 56.7; H, 5.3; N, 9.4; $C_{21}H_{23}N_3O_8$ requires C, 56.6; H, 5.2; N, 9.4); $[\alpha]_D^{27}$+7.9, t.l.c. - D, Rf 0.6, ν_{max} (CH_2Cl_2) 3420 (NH), 3040 (aromatic, CH), 2980 (satd.CH), 1730, 1720 (CO, urethane, acid), 1605, 1595 (aromatic C-C), 1520, 1350 (NO_2), 1280, 1245, 1220, 1090 (C-O) and 860 cm^{-1} (p-subst.benzene); λ_{max} 274 (ε23200) 219nm, 208nm; δ_H ($CDCl_3$, 200 MHz), 8.16 (4H, d, J_{AB} 8.8 Hz), 7.39 (4H, d, J_{AB} 8.8 Hz), 4.67 (3H, m_{AB_2}, $CHCH_2$ Bnpeoc), 4.27 (1H, m, αCH, Ile), 1.9 (1H, m, βCH, Ile), 1.23 (2H, m, γCH_2, Ile), 0.88 (6H, m_{AB}, 2 x CH_3, Ile); δ_C ($CDCl_3$, 50 MHz) 176.1 (CO, acid), 155.8 (CO, urethane), 147.2, 146.8 (quaternary aromatic C's), 129.1, 123.9 (aromatic CH's), 66.0 (CH_2, Bnpeoc), 58.8 (αCH, Ile), 49.8 (CH, Bnpeoc), 37.4 (βCH, Ile), 24.8 (γCH_2, Ile), 15.4, 11.3 (2 x CH_3); m/z (FAB) HRMS, Found: 446.15634; $C_{21}H_{24}N_3O_8$ requires 446.15632 (<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-isoleucine dicyclohexylammonium salt (*121*)

Bnpeoc Ile-OH, DCHA prepared by general procedure of 3.4.1 with (*49*) (0.54 g, 1.2 mmol) in dichloromethane (5 ml). Yield 80%, crystallised from dichloromethane/diethyl ether, m.p. 160-162°C, (Found: C, 63.3; H, 7.50; N, 8.86; $C_{23}H_{46}N_4O_8$ requires C, 63.3; H, 7.35; N, 8.95); $[\alpha]_D^{27}$+7.7; t.l.c. - D, Rf as for (*49*); δ_H ($CDCl_3$, 200 MHz) 8.16 (4H,

d, J_{AB} 8.7 Hz), 7.39 (4H, d, J_{AB} 8.7 Hz), 5.56 (1H, d, J 7.8 Hz), 4.6 (3H, m_{AB_2}, $CHCH_2$-Bnpeoc), 3.9 (1H, m, αCH, Ile), 2.9 (2H, m, 1CH-DCHA), 2.0-0.5 (29H, m, 20H-DCHA, β,γ-CH_2, β,δ-CH_3, Ile); δ_c ($CDCl_3$, 50 MHz) 175.1 (CO, acid), 155.3 (CO, urethane), 147.2 (quaternary aromatic C's x 2), 129.1, 123.8 (aromatic CH's), 65.4 (CH_2, Bnpeoc), 60.6 (αCH, Ile), 52.7 (1CH, DCHA), 50.0 (CH, Bnpeoc), 38.6 (βCH_2, Ile), 29.4 (CH_2, DCHA), 25.1 (CH_2, DCHA), 24.7 (CH_2, DCHA), 15.6, 11.7 (2 x CH_3, Ile); m/z (FAB) HRMS, Found: 627.33937; $C_{33}H_{47}N_4O_8$ requires 627.33936 (<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-leucine (*50*)

Bnpeoc leu-OH was prepared by Route 1.

Yield 90%, recrystallised from diethyl ether, m.p. 67-71°C; (Found: C, 56.9; H, 5.48; N, 9.12; $C_{21}H_{23}N_3O_8$ requires: C, 56.9; H, 5.48; N, 9.44); $[\alpha]_D^{27}$-4.1; t.l.c. - D, Rf 0.5; ν_{max} (CH_2Cl_2) 3420 (NH), 3030 (unsatd.CH), 2980 (satd.CH), 2880 (satd.CH), 1720 (CO, urethane, acid), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1220, 1110, 1080, 1050 (C-O) and 870 cm^{-1} (p-disubst.benzene); λ_{max} 275 (ε17400); δ_H ($CDCl_3$, 200 MHz) 8.16 (4H, d, J_{AB} 8.8 Hz), 7.40 (4H, d, J_{AB} 8.8 Hz), 4.70 (3H, m_{AB_2}, $CHCH_2$-Bnpeoc), 4.27 (1H, m_{B_2X}, αCH, Leu) 1.6 (3H, m_{AB_2}, βCH_2 γCH-leu), 0.91 (6H, d_{AC_6} J 5.5 Hz, 2 x CH_3, leu); δ_c ($CDCl_3$, 50 MHz) 177.2 (CO, acid), 155.5 (CO, urethane), 147.1, 146.7 (quaternary aromatic C's), 129.1, 123.8 (aromatic CH's, 65.7; m/z (FAB), HRMS, Found: 446.15629, $C_{21}H_{24}N_3O_8$ requires 446.1563 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-methionine (*62*) Bnpeoc Met-OH.

Preparation of (*62*) was achieved *via* Route 1. Mixing was however, achieved using a nitrogen line bubbling through the sodium carbonate solution. Work-up was as Route 1 except that extraction with ether and ethyl acetate was enabled using a nitrogen line into the appropriate separation funnel. After drying through Na_2SO_4 and removal of the solvent *in vacuo* a yellow solid was obtained from light petroleum (b.p. 40-60°C) (85%); $[\alpha]_D^{27}$+8.1; t.l.c. - D, Rf 0.4; ν_{max} (CH_2Cl_2) 3420 (NH), 3020 (aryl CH), 2970, 2960 (alkyl CH), 1520, 1350 (NO_2), 1420, 1280, 1225, 1110-1050 (C-O) and 860 cm^{-1} (p-disubst.benzene); λ_{max} 274 (ε15500), δ_H [$(CD_3)_2CO$, 200 MHz] 8.22 (4H, d, J_{AB} 8.8 Hz), 7.70 (4H, d, J_{AB} 8.8 Hz), 4.77 (3H, m_{AB_2} $CHCH_2$-Bnpeoc), 4.30 (1H, m, αCH, Met), 2.5 (2H, m, β-CH_2, Met), 2.0 (5H, m, γCH_2, S-CH_3, Met); δ_C [$(CD_3)_2CO$, 50 MHz] 172.1 (CO, acid), 155.1 (CO, urethane), 147.3, 146.4 (quaternary aromatic C's), 128.9, 122.9 (aromatic CH's), 64.8 (CH_2, Bnpeoc), 52.2 (αCH, Met), 49.0 (CH, Bnpeoc), 30.3 (βCH_2-Met), 29.1 (γCH_2, Met), 13.4 (CH_3, Met); m/z (FAB) HRMS, 464.11272.
$C_{20}H_{22}N_3O_8S$ requires 464.1127 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-methionine dicyclohexylammonium salt (*64*); Bnpeoc MetOH,DCHA

Obtained *via* the general procedure of 3.4.1 with (*62*) (0.94 g, 2.3 mmol) in methanol (2 ml) to give a crystalline precipitate, m.p. 179-180.5, (Found: C, 59.3; H, 6.85; N, 8.72; $C_{32}H_{44}N_4O_8S$ requires: C, 59.6; H, 6.83, N, 8.70;

$[\alpha]_D^{27}$+7.4; m/z (FAB), HRMS, Found: 645.29577; $C_{32}H_{45}N_4O_8S$, requires: 645.29579 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-norvaline (*51*)

Bnpeoc NVa-OH was prepared by Route 1.

Yield 75%; amorphous powder from light petroleum (b.p. 40-60°C), m.p.<25°C; (Found: C, 55.3; H, 5.12; N, 9.75; $C_{20}H_{21}N_3O_8$ requires: C, 55.7; H, 4.87; N, 9.70); $[\alpha]_D^{27}$ -1.3, t.l.c. - D, Rf 0.5; ν_{max} (CH_2Cl_2) 3425 (NH), 2970 (alkyl, CH), 1720 (CO urethane, acid), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1420, 1240, 1225, 1110 (C-O) and 860 cm^{-1} (p-disubst.benzene); λ_{max} 274 (ε 19840), δ_H ($CDCl_3$, 200 MHz) 8.15 (4H, d, J_{AB} 8.6 Hz), 7.39 (4H, d, J_{AB} 8.6 Hz), 4.70 (3H, m_{AB_2}, $CHCH_2$-Bnpeoc), 4.25 (1H, m, αCH), 1.70 (2H, m, βCH_2), 1.30 (2H, m, γCH_2), 0.87 (3H, m, δCH_3); δ_c ($CDCl_3$, 50 MHz) 176.6 (CO, acid), 155.5 (CO, urethane), 147.1, 146.8 (quaternary aromatic C's), 129.1, 123.9 (aromatic CH's), 65.9 (CH_2, Bnpeoc), 53.6 (αCH), 49.8 (CH, Bnpeoc), 34.0 (βCH_2), 18.4 (γCH_2), 13.3 (δCH_3); m/z (FAB), Found: 432.14069, $C_{20}H_{22}N_3O_8$ requires; 432.14068 (.˙. = <1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-phenylalanine (*52*)

Bnpeoc Phe-OH was prepared by Route 1.

Yield 67%; crystallised from diethyl ether, recrystallised from chloroform/diethyl ether; $[\alpha]_D^{27}$ -15.7; t.l.c. - D, Rf 0.6; ν_{max} (CH_2Cl_2) 3420 (NH), 3020 (aryl CH), 2980 (alkyl CH), 1730 (CO, urethane, acid), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1420, 1250, 1110, 1080, 1050 (C-O), 880

and 860 cm^{-1}; λ_{max} 274 (ε22000); δ_H (CD_2Cl_2, 200 MHz) 8.17 (4H, d, J_{AB} 8.8 Hz), 7.41 (4H, d, J_{AB} 8.8 Hz), 7.26 (3H, m, Phe), 7.15 (2H, m, Phe), 4.61 (4H, m, $CHCH_2$ Bnpeoc, αCH Phe), 3.12 (2H, m_{ABX}, β CH_2 Phe); m/z (FAB) HRMS; Found: 480.14065; $C_{24}H_{22}N_3O_8$ requires 480.1407 (.˙.<1 ppm).

H.P.L.C., A (100%) - B (100%) 25 min, RT = 22 min;
A:B (50:50) - B (100%) 25 min, RT = 11 min.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-proline (*53*)

Bnpeoc Pro-OH was prepared by Route 1.

Yield 75%; amorphous powder from light petroleum (b.p. 40-60°C); (Found: C, 56.3; H, 4.4; N, 9.56; $C_{20}H_{18}N_3O_8$ requires: C, 55.9; H, 4.8; N, 9.5); $[\alpha]_D^{27}$ -18.7; t.l.c. - C, Rf 0.2; ν_{max} (CH_2Cl_2) 3040 (aryl CH), 2960 (alkyl CH), 1760, 1705 (CO, urethane, acid), 1605, 1595 (C-C aromatic), 1520, 1350 (NO_2), 1420, 1280, 1240, 1185, 1130, 1110 (C-O), 860 cm^{-1}; λ_{max} 274 (ε19700), 220 nm, 205 nm, δ_H ($CDCl_3$, 200 MHz) 8.20 (4H, m, 4 x aromatic CH_A's); 7.40 (4H, m, 4 x aromatic CH_B's), 4.66 (3H, m, $CHCH_2$ Bnpeoc), 4.37 (½H, m, αCH, Pro), 4.12 (½H, m, αCH, Pro), 3.4 (2H, m, δCH_2), 2.0 (4H, m, γCH_2, Pro); δ_c ($CDCl_3$, 50 MHz) 176.4 (acid CO x 2), 156.7, 154.5 (CO, urethane), 147.1, 146.9 (quaternary aromatic C's), 129.1, 123.9 (aromatic CH's), 66.3 (CH_2, Bnpeoc), 58.9, 58.3 (αCH, Pro), 49.6 (CH, Bnpeoc), 46.8, 46.3 (δCH_2, Pro), 24.0, 23.1 (γCH_2, Pro); m/z (FAB) HRMS; Found: 430.1250: $C_{20}H_{20}N_3O_8$ requires 430.1250 (.˙.<1 ppm).

N-2,2-Bis[(4-nitrophenyl)ethoxycarbonyl]-L-proline dicyclohexylamine salt (*122*)

Bnpeoc Pro OH.DCHA salt prepared as described in Sect.3.4.1 crystallised from dichloromethane/diethyl ether; m.p. 94-96°C; $[\alpha]_D^{27}$ -13.6; m/z (FAB) HRMS; Found: 611.30809: $C_{32}H_{43}N_4O_8$ requires: 611.30807 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-serine (*54*)

Bnpeoc Ser-OH was prepared by Route 1.

Yield 78%; recrystallised from acetone/light petroleum (b.p. 40-60°C); m.p. 169-173°C; (Found: C, 51.30; H, 4.06; N, 9.92; $C_{18}H_{17}N_3O_9$ requires: C, 51.70; H, 3.83; N, 10.10); $[\alpha]_D^{27}$ +3.1; t.l.c. - D, Rf 0.2; ν_{max} (Bromoform mull) 3480 (OH), 3200 (NH), 1750, 1720, 1660 (CO), 1605, 1595 (C-C, aromatic), 1560, 860, 830, 750, 700 and 650 cm^{-1}; λ_{max} 274 (ε19650); δ_H [$(CD_3)_2CO$, 200 MHz], 8.21 (4H, d, J_{AB} 8.8 Hz), 7.69 (4H, d, J_{AB} 8.8 Hz), 6.42 (1H, m, NH), 4.85 (3H, m_{AB_2}, $CHCH_2$-Bnpeoc), 4.28 (1H, m_{ABC}, αCH), 3.93 (1H, dd, J_{AB} 11 Hz, J_{AC} 4 Hz), 3.83 (1H, dd, J_{AB} 11 Hz, J_{BC} 4 Hz); δ_C [$(CD_3)_2CO$, 50 MHz] 170.4 (CO, acid), 155.0 (CO, urethane), 147.3, 146.5 (quaternary aromatic C's), 128.9, 123.1 (aromatic CH's), 65.0 (CH_2, Bnpeoc), 61.2 (CH_2, Ser), 55.5 (αCH) 49.0 (CH, Bnpeoc); m/a (FAB) HRMS; Found: 420.10428; $C_{18}H_{18}N_3O_9$ requires: 420.1043 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-threonine (*55*)

Bnpeoc Thr-OH was prepared by Route 1

Yield 85%; recrystallised from acetone/light petroleum (b.p. 40-60°C); m.p. 171-173°C; (Found: C, 53.0; H, 4.41; N, 9.78; $C_{19}H_{19}N_3O_9$ requires: C, 52.7; H, 4.39; N, 9.70); $[\alpha]_D^{27}$ +3.8; t.l.c. - D, Rf 0.2; ν_{max} (Bromoform mull) 3620 (NH), 3405 (OH), 1765, 1750, 1670, 1660 (CO), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1550, 1325, 1280, 1200, 1150, 1105, 1075 (C-O), 870, 860 and 835 cm^{-1} (aryl); λ_{max} 275 (ε20000); δ_H [$(CD_3)_2CO$, 200 MHz] 8.21 (4H, d, J_{AB} 8.8 Hz), 7.70 (4H, d, J_{AB} 8.8 Hz), 6.15 (1H, m, NH), 4.78 (3H, m, $CHCH_2$Bnpeoc), 4.30 (1H, m, αCH), 4.19 (1H, m, βCH, Thr), 1.15 (3H, d, J 6.4 Hz, γCH_3, Thr); δ_C [$(CD_3)_2CO$, 50 MHz] 170.61 (CO, acid), 155.4 (CO, urethane), 147.2, 146.5 (quaternary aromatic C's), 128.9, 122.9 (aromatic CH's), 66.2 (β-CH, Thr), 65.0 (CH_2, Bnpeoc), 58.7 (αCH), 49.0 (γCH_3, Thr); m/z (FAB) HRMS; Found: 434.11991: $C_{19}H_{20}N_3O_9$ requires 434.1199 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-tryptophan (*63*)

Bnpeoc Trp-OH was prepared by Route 1, modified as for Bnpeoc Met-OH (*62*)

Yield 60%; recrystallised from chloroform; m.p. 76-82°C; t.l.c. - C, Rf 0.1; D, Rf 0.5; ; ν_{max} (nujol mull) 3450 (NH x 2), 1730 (CO, urethane), 1670 (acid), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1240, 1080, 860 and 830 cm^{-1}; λ_{max} 275 (ε24000); δ_H [$(CD_3)_2CO$, 200 MHz) 8.18 (4H, d, J_{AB} 8.6 Hz), 7.65 (5H, m, 4H Bnpeoc, 1H, Trp),

7.40 (1H, d, J 8.1 Hz, 2CH, Trp), 7.10 (3H, m, Trp), 4.68 (3H, m, $CHCH_2$, Bnpeoc), 4.6 (1H, m, αCH), 3.26 (2H, m, CH_2, Trp); m/z (FAB) HRMS; Found: 519.15155; $C_{26}H_{23}N_4O_8$ requires: 519.1515 (.˙.<1 ppm); H.P.L.C. A:B (50:50) - B (100%) 25 min, Rt = 11 min.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]tryptophan dicyclohexylammonium salt (*65*), see 3.4.1.

M.p. 162-164 (D), (Found: C, 65.7; H, 6.3; N, 10.0; $C_{38}H_{44}N_5O_8$ requires: C, 65.3; H, 6.56; N, 10.1; $[\alpha]_D^{27}$ -1.5; λ_{max} 275 (ε24000); δ_H [$(CD_3)_2CO$, 200 MHz] 8.1 (4H, m), 7.6-6.8 (9H, m), 4.5 (4H, m, $CHCH_2$, Bnpeoc, αCH Trp), 3.25 (2H, m, βCH_2 Trp), 2.9 (2H, m, 1CH x 2 DCHA), 2.3-0.75 (20H, m, DCHA); δ_c ($CDCl_3$, 50 MHz) 175.5 (CO, acid), 155.0 (CO, urethane), 147.1, 147.0 (quaternary aromatic C's, Bnpeoc), 136.0 (quaternary C9, Trp), 123.7 (aromatic CH, Bnpeoc), 122.5 (C2-Trp), 121.6 (C5-Trp), 118.8 (C4, C6-Trp), 111.8 (C7-Trp), 110.9 (C3-Trp), 65.3 (CH_2, Bnpeoc), 56.3 (αCH, Trp), 52.6 (1CH, DCHA), 49.6 (CH, Bnpeoc), 29.2 (CH_2, DCHA), 24.9 (CH_2, DCHA), 24.5 (CH_2, Bnpeoc); m/z (FAB) HRMS; Found: 700.33463, $C_{38}H_{46}N_5O_8$ requires: 700.334615 (.˙.<1 ppm).

N-[2,2]Bis(4-nitrophenyl)ethoxycarbonyl]-L-tyrosine (*67*) Bnpeoc Tyr-OH, was prepared by Route 2

Work-up of the reaction after 1 hour stirring at room temperature gave a foam (1.8 g, 67%), silica gel chromatography (chloroform, 1:99) removed a small impurity to provide

(*67*); white amorphous powder from light petroleum (b.p. 40-60°C); (Found: C, 56.7; H, 4.39; N, 8.21; $C_{24}H_{21}N_3O_9 \cdot \frac{1}{2}H_2O$ requires: C, 57.1; H, 4.37; N, 8.33); $[\alpha]_D^{27}$ -14.5; t.l.c. - D, Rf 0.2; ν_{max} (CH_2Cl_2) 3580 (OH), 3420 (NH), 1730, 1720 (CO, urethane, acid), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1420, 1240, 1105, 1050 (C-O), 860 and 830 cm^{-1} (aryl); λ_{max} 274 (ε19000); δ_H [$(CD_3)_2CO$, 200 MHz] 8.21 (4H, d, J_{AB} 8.7 Hz), 7.66 (4H, d, J_{AB} 8.7 Hz), 7.05 (2H, d, J_{AB} 8.4 Hz, Tyr), 6.75 (2H, d, J_{AB} 8.4 Hz, Tyr), 4.70 (3H, m, $CHCH_2$, Bnpeoc), 4.40 (1H, m,α CH), 3.0 (2H, m_{ABC}, benzyl CH_2, Tyr); δ_c [$(CD_3)_2CO$, 50 MHz] 171.6 (CO, urethane), 155.4 (CO, acid), 154.8 (quaternary aromatic C, phenolic), 147.1 (quaternary aromatic, Bnpeoc), 146.2 (quaternary aromatic, Bnpeoc), 129.4 (aromatic CH, Tyr), 128.8 (aromatic CH, Bnpeoc), 127.0 (quaternary aromatic, Tyr), 122.8 (aromatic CH, Bnpeoc), 114.3 (aromatic CH, Tyr), 64.8 (CH_2, Bnpeoc), 54.6 (αCH, Tyr), 48.8 (CH, Bnpeoc), 35.6 (βCH_2, Tyr); m/z (FAB) HRMS: Found: 496.13561; $C_{24}H_{22}N_3O_9$ requires 496.1356 (.˙.<1 ppm).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-tyrosine dicyclohexylammonium salt (*123*)

This was obtained through general procedure of 3.4.1 using (*67*) (0.64 g, 1.3 mmol) in dichloromethane (4 ml). Removal of the solvent afforded a residue which was crystallised from acetonitrile (0.75 g, 85%); m.p. 171-173°C; (Found: C, 64.2; H, 6.68; N, 8.23; $C_{36}H_{44}N_4O_9$

requires: C, 63.9; H, 6.5; N, 8.3); $[\alpha]_D^{27}$ +25.6.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-valine (*56*)

Bnpeoc Val-OH was prepared by Route 1.

Yield 75%; recrystallised from ethyl acetate/light petroleum (b.p. 40-60°C); m.p. 148-150°C; (Found: C, 55.8; H, 4.65; N, 9.77; $C_{20}H_{21}N_3O_8$ requires: C, 55.8; H, 4.91; N, 9.57); $[\alpha]_D^{27}$ +6.2; t.l.c. - D, Rf 0.4; ν_{max} (CH_2Cl_2) 3430 (NH), 2970 (CH), 1730, 1720 (CO, urethane, acid), 1695, 1595, 1520, 1350 (NO_2), 1420, 1220, 1110, 1100 C-O) and 860 cm^{-1}; λ_{max} 274 (ε23000); δ_H [$(CD_3)_2CO$, 200 MHz] 8.21 (4H, d, J_{AB} 8.7 Hz), 7.69 (4H, d, J_{AB} 8.7 Hz), 4.8 (3H, m, $CHCH_2$-Bnpeoc), 4.1 (1H, m, αCH), 2.1 (1H, m, βCH, Val), 0.9 (6H, m, 2 x CH_3), δ_c [$(CD_3)_2CO$, 50 HMHz] 171.5 (CO, acid), 155.2 (CO, urethane), 147.3, 146.4 (quaternary aromatic C's), 128.9, 122.8 (aromatic CH's), 64.9 (CH_2, Bnpeoc), 58.5 (αCH), 49.0 (CH, Bnpeoc), 29.6 (βCH, Val), 17.7, 16.4 (CH_3 x 2); m/z (FAB) HRMS; Found: 432.14069; $C_{20}H_{22}N_3O_8$ requires 432.14068 (.˙.<1 ppm).

3.5 PREPARATION OF BNPEOC-PEPTIDES IN SOLUTION

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]alanyl phenylalanyl glycine methyl ester (*70*)

Bnpeoc Ala Phe Gly OMe

Bnpeoc Ala-OH (1.0 g, 1.5 mmol) was suspended in dry dichloromethane (40 ml) and cooled to -10°C. To this was added diphenylphosphinyl chloride(*124*) [(2.5 mmol, 8.6 ml of 0.7 g of (*124*)in dichloromethane (10 ml)] and N-methylmorpholine (0.24 ml, 2.5 mmol) resulting in a clear solution

To this was added phenylalanylglycine methyl ester trifluoroacetate (*69*) (0.75 g, 1.15 mmol) in DMF (5 ml) at such a rate that the temperature did not rise above -5°C. After complete addition NMM (0.25 ml, 2.25 mmol) and 2,6-Lutidine (0.26 ml, 2.25 mmol) were added. The mixture was allowed to stir for 1 hour at 0°C and for 1 hour at room temperature (12°C) before the solvent was removed *in vacuo* to produce a crystalline/gum matrix. The residue was partitioned between ethyl acetate/water from which the organic layer was washed with $NaHCO_3$ (2 x 20 ml), water, brine and finally dried over Na_2SO_4. Chromatography on silica gel ($CHCl_3$) provided pure (*70*) crystallised from ethyl acetate/light petroleum (b.p. 40-60°C); 1 g, 75%; m.p. 159-162°C; (Found: C, 57.7; H, 4.92; N, 11.2; $C_{30}H_{31}N_5O_{10}$ requires; C, 57.9; H, 5.18; N, 11.2): t.l.c. - D, Rf 0.3; $[\alpha]_D^{27}$ -3.9; ν_{max} (CH_2Cl_2) 3420 (NH), 3980, 3050 (CH, aryl), 1750, 1730, 1675 (CO, urethane, amide), 1605, 1595 (C-C, aromatic), 1520, 1350 (NO_2), 1440 and 1420 cm^{-1}; λ_{max} 274 (ε21000); δ_H ($CDCl_3$, 200 MHz) 8.2 (4H, d, J_{AB} 8.7 Hz), 7.4 (4H, d, J_{AB} 8.7 Hz) (aromatic Bnpeoc CH's), 7.2 (5H, m, Phe, CH's), 6.8 (1H, d, J 7.9 Hz, urethane NH), 6.6 (1H, m, NH, Gly), 5.3 (1H, d, J 7.2 Hz, amide NH), 4.6 (4H, m, $CHCH_2$, Bnpeoc, αCH Phe), 4.2 (1H, αCH, Ala), 4.0 [1H, dd, J_{AB} 14 Hz (CH_A Gly) J^2 6 Hz (NH Gly), CH_B Gly], 3.8 ([1H, dd, J_{AB} 14 Hz (CH_B Gly) J 6 Hz (NH Gly), CH_A Gly], 3.7 (3H, s, OMe), 3.0 (2H, d, J 6 Hz, CH_2, Phe), 1.3 (3H, d, J 5 Hz, CH_3 Ala); δ_c ($CDCl_3$, 50 MHz) 171.8, 170.6, 169.6, 155.1 (CO), 147.1, 146.8, 136.1 (quaternary

aromatic CH's), 129.1, 128.5, 126.9, 123.9 (aromatic CH's), 66.0 (CH_2, Bnpeoc), 54.0, 52.2, 50.6, 49.6 (CH x 2, OCH_3, Bnpeoc CH), 41.0, 38.2 (CH_2, Phe, Gly), 18.4 (CH_3, Ala); m/z (FAB) HRMS: Found: 622.2149; $C_{30}H_{31}N_5O_{10}$ requires 622.2149.

Amino Acid Analysis: Gly, 1.04; Ala, 0.96; Phe, 1.10;

H.P.L.C. A:B (50:50) - B (100%) 25 min, RT = 11 min.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]aminoisobutyl alanine methyl ester (*73*)

A n.m.r. investigation into the thermal stability and coupling efficiency of 1-oxophospholane mixed anhydride. 1-Chloro-1-oxophospholane (Cpt-Cl) (0.15 g, 1.05 mmol) was dissolved in a 1 ml volumetric flask under anhydrous conditions with dry dichloromethane. From this solution was withdrawn 110 µl (15.3 mg, 0.11 mmol) and added to a n.m.r. tube containing Bnpeoc Aib-OH (*61*) (0.046 g, 0.11 mmol) in DMF (0.5 ml) at 0°C. N-Methylmorpholine (12.1 µl, 0.11 mmol) was added and the tube shaken until a white precipitate was observed. The n.m.r. tube was then placed in the probe and ^{31}P spectra were obtained at 30°C for ten minutes. After 14 minutes alanine methyl ester hydrochloride (*72*) (15.4 mg, 0.11 mmol) in DMF (0.25 ml) and NMM (25 µl, 0.22 mmol) were added at 0°C. Spectra were obtained at appropriate intervals whilst the temperature was kept at 30°C for 10 min after addition then at 47°C for 10 min and finally at 55°C for 157 min after the addition. The n.m.r.

reaction was allowed to stand in a sonic bath for 18 hours after which time the solvent was removed *in vacuo* and the residue partitioned between ethyl acetate and water. The organic phase was washed with $NaHCO_3$, water, 5% citric acid, water and dried over Na_2SO_4. The solvent was removed *in vacuo* to produce a gum which was purified on a silica preparative t.l.c. plate (CH_3Cl_3/MeOH, 1:99) to afford two products; t.l.c. - C, Rf 0.4 (16 mg), 0.75 (17 mg).

Product at Rf (0.4).

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]aminoisobutyl alanine methyl ester (*73*). δ_H ($CDCl_3$, 200 MHz) 8.19 (4H, d, J_{AB} 8.2 Hz), 7.39 (9H, d, J_{AB} 8.2 Hz), 6.6 (1H, m, NH amide), 5.33 (1H, m, NH, urethane), 4.63 (4H, m, $CHCH_2$-Bnpeoc, αCH, Ala], 3.75 (3H, s, OCH_3), 1.5 (9H, m, 2 x CH_3-Aib, CH_3-Ala); δ_C ($CDCl_3$, 50 MHz, DEPT) 129.0, 123.9 (aromatic CH's), 65.6 (CH_2, Bnpeoc), 52.4 (OCH_3), 49.6 (CH, Bnpeoc), 48.1 (αCH, Ala), 25.0 (2 x CH_3, Aib), 18.1 (CH_3, Ala).

^{31}P spectra	δ	δ
(a) Cpt-Cl/CH_2Cl_2	88.31	
(b) Bnpeoc Aib-Cpt.	76.65	
(c) 10 min at 30°C	76.65	
Addition of HCl Ala OMe (*72*)		
(e) t = 2 min (30°C)	76.65	
(f) t = 10 min (30°C)	76.65	
(g) t = 20 min (47°C)	76.25	64.38 (v.small)

	δ	δ
(h) t = 32 min (55°C)	76.05	65.18 (small)
(i) t = 80 min (55°C)	76.05	65.39 (medium)
(j) t = 120 min at 55°C	76.05	65.59 (½ peak height)
(k) t = 14 h/sonic bath	75.45	64.58 (large)

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-alanine benzylamide (*78*)

Prepared for a study into the nucleophilicity of a primary amine (benzylamine) with relation to basicity in presence of β-eliminating protecting groups

To a suspension of Bnpeoc Ala (*44*) (0.23 g, 0.58 mmol) in dry DCM (8 ml) at -6°C was added diphenylphosphinyl chloride (0.5 mmol in 3.2 ml DCM) and NMM (63 µl, 0.58 mmol). A clear solution formed and 2,6-lutidine (67µl, 0.58 mmol) followed by benzylamine (*76*) (42 µl, 0.38 mmol) were added at -6°C. After 30 minutes stirring at room temperature the solvent was removed and the residue partitioned between ethyl acetate/water. The organic phase was washed with $NaHCO_3$, water, citric acid, water, brine and finally dried through Na_2SO_4. The resulting solution was reduced *in vacuo* to produce a foam. H.P.L.C. analysis of the crude product showed that no olefinic product had been formed in the coupling reaction. This was confirmed by repeating the H.P.L.C. with the crude product 'spiked' with olefin (*28*). H.P.L.C. A:B (50:50) - B (100%) 25 min, Bnpeoc Ala-OH, RT = 7 min, crude product (as foam) RT = 7 min (1905); RT = 11 min (8639, benzylamide (*76*)); RT = 13 min

(651); 'Spiked' Crude Product RT = 7 min (1555), RT = 11 min (7101), RT = 13 min (537), RT = 15 min (6981, olefin).

The crude product was then crystallised from diethyl ether/chloroform, H.P.L.C. A:B (50:50) - B (100%) 25 min RT = 11 min, ν_{max} (CH_2Cl_2) 3440 (NH), 1730 (CO, urethane), 1680 (CO, amide), 1605, 1595 (C-C, aromatic), 1520 and 1350 cm^{-1} (NO_2); δ_H ($CDCl_3$, 200 MHz) 8.15 (4H, d, J_{AB} 8.6 Hz), 7.34 (4H, d, J_{AB} 8.6 Hz), 7.23 (5H, m, CH x 5, benzyl), 6.4 (1H, m, amide), 5.49 (1H, d, J 7 Hz, NH urethane), 4.6-4.13 (6H, m, $CHCH_2$-Bnpeoc, CH_2-benzyl, αCH-Ala), 1.33 (3H, d, J 6.8 Hz); δ_C ($CDCl_3$, 50 MHz) 171.6 (CO, amide), 155.1 (CO, urethane), 147.2, 146.7 (quaternary aromatic Bnpeoc), 137.6 (quaternary aromatic benzyl), 131.6, 129.0, 128.6, 127.5, 123.9 (aromatic CH's), 65.9 (CH_2, Bnpeoc), 50.6 (αCH), 49.7 (CH, Bnpeoc), 43.5 (CH_2, benzyl), 18.7 (CH_3, Ala); m/z (FAB); Found: 493.17233; $C_{25}H_{25}N_4O_7$ requires 493.17231 (∴<1 ppm). Mp 128-131°C (D)

3.5.1 Preparation of Fmoc derivatives

N-[9-fluorenylmethoxycarbonyl]-L-alanine benzylamide (79)

The above procedure was repeated with N-[9-fluorenylmethoxycarbonyl]-L-alanine (*77*)(0.17 g, 0.58 mmol) and diphenylphosphinylchloride (0.58 mmol in 3.1 ml DCM), to which was added NMM (63 µl, 0.58 mmol) 2,6-lutidine (6.7 µl, 0.58 mmol) and benzylamine (43 µl, 0.38 mmol). The reaction was worked-up to give a gum (0.22 g) on removal of the solvent *in vacuo*. T.l.c. analysis of crude product showed no olefin (*28*) formation. T.l.c. - B, Rf (olefin) 0.6;

t.l.c. - B (crude product) Rf 0.2. The crude product was crystallised from diethyl ether/chloroform; ν_{max} (CH_2Cl_2) 3440 (NH), 1740 (CO, urethane), 1690 (CO, amide), 1510 (C-C, aryl), 1440 (CH) and 1200-1100 cm^{-1} (C-O); δ_H ($CDCl_3$, 200 MHz) 7.8-7.2 (13H, 5H aryl, 8H fluorene), 4.25 (6H, m, $CHCH_2$ (Fmoc), αCH, Ala, CH_2 benzyl); 1.39 (3H, d, J 6.9 Hz, CH_3 Ala); δ_c ($CDCl_3$) 172.1 (CO, amide), 143.7, 141.25 (quaternary C's on fluorene), 137.8 (quaternary C, benzyl), 131-121 (13 aromatic CH's), 67.0 (CH_2,Fmoc), 50.7 (αCH, Ala), 47.1 (CH, Fmoc), 43.5 (CH_2, benzyl), 18.5 (CH_3, Ala); m/z (FAB) 401, 240, 219, 179; Found: MH^+ 401.18600, calc. for $C_{25}H_{25}N_2O_3$ 401.18650 (.˙.<2 ppm); H.P.L.C. A:B (50:50) - B (100%) 25 min, RT = 12 min.
Mp 175-180°C

9-Fluorenylmethyl acetate (*75*)

Acetic anhydride (0.36 ml, 3.84 mmol) was added to 9-fluorenylmethanol (*125*) (0.63 g, 3.2 mmol) in pyridine (5 ml) and was stirred for eighteen hours. The solvent was removed *in vacuo* to produce 9-fluorenylmethyl acetate (*75*) as a white solid, recrystallised from chloroform.
δ_H ($CDCl_3$, 200 MHz) 7.5 (8H, m, CH's fluorenyl), 4.35 (2H, d_{AB_2}, CH_2), 4.18 (1H, m_{AB_2}, CH), 2.15 (3H, s, CH_3);
δ_c ($CDCl_3$, 50 MHz) 170.7 (CO, acetate), 143.8, 141.3 (quaternary C's), 127.7, 126.9, 124.9, 119.9 (aromatic CH's), 66.3 (CH_2), 46.8 (CH), 20.8 (CH_3); H.P.L.C. A:B (50:50) - B (100%) 25 min, RT = 13 min; m/z (FAB) HRMS: Found: 239.107211; $C_{16}H_{15}O_2$ requires 239.10720 (.˙.<1 ppm)
Mp 84-85°C

3.6 CALIBRATION OF H.P.L.C. AND KINETICS FOR ELIMINATION STUDIES

General Method

2,2-Bis(4-nitrophenyl)ethylcinnamate (*29*) (2 mg, 4.8 mmol) was dissolved in acetonitrile (2 ml) 1 µl was withdrawn from this solution and injected into the H.P.L.C. After the peak had been recorded, the base line allowed to settle, another volume of standard was injected. This procedure was repeated with 2 µl, 5 µl, 7 µl, 10 µl, 15 µl and 25 µl. Results for the olefin (*28*), acetate (*8*) were obtained in this manner.

Table 3.1

1,1-Bis(4-nitrophenyl)ethene (*28*)

moles injected (y) ($x10^8$)	peak area (x)
0.23	732
0.46	1510
1.15	3658
1.6	5264
2.3	7447
3.4	11129
5.7	16999

Table 3.2

2,2-Bis(4-nitrophenyl)ethyl acetate (*8*)

moles injected (y) ($x10^8$)	peak area (x)
0.19	792
0.39	1555
0.97	3893
1.36	5343
1.94	7540
2.9	10902
4.8	16481

Table 3.3

2,2-Bis(4-nitrophenyl)ethyl cinnamate (*29*)

moles injected (y) ($x10^8$)	peak area (x)
0.12	746
0.24	1718
0.84	5622
1.8	11994
2.4	15500
3.0	19125

3.6.1 A TYPICAL EXPERIMENT INTO THE MECHANISM FOR ELIMINATION OF 2,2-BIS(4-NITROPHENYL)ETHYL CINNAMATE (*29*)

(*29*) (0.14 g, 0.35 mmol) dissolved in dichloromethane (25 ml) was placed into a thermostatted tank and allowed to equilibrate with stirring. A stopwatch was started as 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (44 µl, 0.35 mmol) was added to the solution. 0.5 ml samples were withdrawn at appropriate time intervals and 6 drops 1M acetic acid/methanol added to each. The exact time and temperature were then recorded. The residue produced on removal of the solvent *in vacuo* was dissolved in H.P.L.C. grade acetonitrile (2 ml) and homogenised. From this was injected a 20 µl sample into the H.P.L.C. Peak areas are quoted in parentheses after the retention time.

Table 3.4

(i) Elimination of (*29*) at 18°C, with 1 DBN/CH_2Cl_2

Time (seconds)	ester (*29*) peak area	time (seconds)	ester (*29*) peak area
47	23088	701	15919
97	22964	833	16766
207	22790	1073	12939
268	20836	1373	14245
427	19307	2526	2114
597	18008		

(ii) Elimination of (*29*) at 8°C, with 1 DBN/CH_2Cl_2

Time (seconds)	ester (*29*) peak area	time (seconds)	ester (*29*) peak area
76	20241	8759	2340
310	19781	7333	2940
515	17171	4813	5315
1110	13740	1875	8550
1425	12052		

(iii) Elimination of (*29*) at -3°C with 1 DBN/CH_2Cl_2

Time (seconds)	ester (*29*) peak area	time (seconds)	ester (*29*) peak area
299	23027	12307	3916
621	19956	11054	4745
1096	19062	7241	8280
1701	16710	3455	9265
2361	14993		

(iv) Elimination of (*29*) at -17°C with 1 DBN/CH_2Cl_2

Time (seconds)	ester (*29*) peak area	time (seconds)	ester (*29*) peak area
165	17183	10075	7961
1134	15522	8397	6972
1413	16663	11388	6631
1841	15197	12677	4433
2464	13256	15470	2930
3798	12708	17420	5389
4578	12982	18536	2789
7074	7377		

Elimination of (*29*) at -5°C with 2 DBN/CH_2Cl_2

For this reaction the general method described in Section 3.6.1 was followed using cinnamate (*29*) (0.198 g, 0.49 mmol) in dichloromethane (50 ml) and allowed to equilibrate at -5°C. To this was added DBN (0.12 µl, 0.98 mmol, 2 equivs) from which samples of 0.5 ml were withdrawn and analysed as before (Table 3.5).

Table 3.5

Elimination of (*29*) at -5°C with 2 DBN/CH_2Cl_2

Time (seconds)	ester (*29*) peak area	time (seconds)	ester (*29*) peak area
90	13664	5100	5132
351	12056	7473	3538
861	10608	8922	2502
1187	9815	89660	1985
1860	8694	12126	2500
3075	6786	13210	2181
4020	5971		

Elimination of (*29*) at -5°C with 4DBN/CH_2Cl_2

Method as for 2DBN/CH_2Cl_2 using (*29*) (0.197 g, 0.49 mmol) in dichloromethane (50 ml) with DBN (0.24 µl, 1.96 mmol, 4 equivs.) at -5°C. Samples of 0.5 ml taken as appropriate (Table 3.6).

Table 3.6

Time (seconds)	Ester peak area (*29*)
672	8037
1976	4327
2834	2972
3931	1899
5082	1081

3.6.2 Elimination study of 4-nitrophenylethyl cinnamate (*31*) with DBN

(i) The general method (Section 3.6.1) was followed using ester (*31*) (0.093 g, 0.31 mmol) in DCM (20 ml) with DBN (40 µl, 0.31 mmol) at 20°C. Samples of 0.5 ml were withdrawn and analysed by H.P.L.C. and t.l.c. as before.

(ii) This procedure was repeated with ester (*31*) (0.16 g, 0.53 mmol) in DCM (30 ml) with DBN (65.6 µl, 0.53 mmol) at 50°C. Neither of these experiments showed cleavage products by H.P.L.C. or t.l.c.

(iii) Elimination of (*31*) with 1M DBN in pyridine at 20°C. The above procedure was repeated with ester (*31*) (0.16 g,

0.53 mmol) dissolved in 1M DBN in pyridine (10 ml). Samples of 0.5 ml were withdrawn at time intervals over 1 hour. The solvent was then removed to produce a red liquid which was partitioned between dichloromethane and 0.1M NaOH. Acidification of the aqueous phase with di1 HCl (aq) produced a precipitate which was extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ and concentrated *in vacuo* to produce a yellow solid found to be cinnamic acid.

3.7 STABILITY STUDIES

(i) To 2,2-Bis(4-nitrophenyl)ethyl cinnamate (*29*) (0.15 g, 0.37 mmol) in dry dichloromethane (25 ml) was added triethylamine (51 µl, 0.37 mmol). The reaction was followed by t.l.c. for forty-five hours after which time the solvent was removed and the residue analysed. Results obtained by t.l.c. and H.P.L.C. showed no cleavage products after 15 hours although a small trace of olefin was observed after 45 hours; t.l.c. - A (15 hrs) 0.26; t.l.c. - A (45 hrs) 0.26 (major), 0.4 (minor); ν_{max} (CH_2Cl_2) 1710 (CO, ester) 1640, 1605, 1595, 1520, 1350 and 1110 cm^{-1}.

(ii) To a solution of (*29*) (0.1 g, 0.25 mmol) in DMF (5 ml) was added tetrabutylammonium fluoride (trihydrate) (0.24 g, 0.75 mmol) in DMF (5 ml), the total volume being made up to 20 ml by rinsing the reagent vial. On addition a blue colour formed which faded to give a clear solution. After stirring for an hour the solvent was removed *in vacuo* to produce a gum which was partitioned between ethyl acetate and 0.1M NaOH. Acidification of the aqueous phase produced a white precipitate, extracted with ethyl acetate. The combined organic phase was reduced *in vacuo* to produce a yellow solid (45 mg) found to be slightly impure cinnamic acid (*30*); t.l.c. - B, Rf (product) 0.2; t.l.c. - B, Rf (cinnamic acid) 0.2;

(iii) (*29*) (0.12 g, 0.27 mmol) was dissolved in trifluoro-acetic acid (0.5 ml), filtered into a n.m.r. tube and spectra recorded over twenty-four hours. The solvent was then

removed *in vacuo* to produce the ester (*29*) as a white crystalline solid (0.08 g, 69% recovery); t.l.c. - B, Rf 0.52; ν_{max} (CH_2Cl_2) 1710 (CO, ester), 1640, 1605, 1595, 1350, 1520 (NO_2) cm^{-1}.

(iv) (*29*) (0.14 g, 0.34 mmol) was suspended in 3N HCl/ methanol (2 ml, 17 equivalents) and stirred for 18 hours. The solvent was removed, completely after repeated addition of methanol, to produce a residue found by H.P.L.C. to contain only pure (*29*). H.P.L.C. A:B (50:50) - B (100%) 28 min, RT = 13 min.

(v) (*29*) (0.12 g, 0.29 mmol) dissolved in pyridine (15 ml) was stirred for twenty-four hours at room temperature. Removal of the solvent, after repeated addition of toluene, produced a residue confirmed by t.l.c. and i.r. to be the ester (*29*) with no elimination products; t.l.c. - B, Rf 0.52; t.l.c. - B of (*29*), Rf 0.52; ν_{max} (CH_2Cl_2) 1715 (CO), 1605, 1595, 1520, 1350 cm^{-1}.

(vi) To (*29*) (0.259 g, 0.65 mmol) in ethyl acetate (10 ml) was added 10% Pd/c (0.025 g) and allowed to stir under a hydrogen atmosphere. Samples were withdrawn at appropriate intervals and analysed by t.l.c. After two hours the reaction was filtered through celite and the solvent removed *in vacuo*. The products from this reaction were separated by silica chromatography [ethyl acetate/light petroleum (b.p. 40-60°C) 1:20-1:5]. 2,2-Bis(4-nitrophenyl)ethyl dihydrocinnamate (*83*), t.l.c. - C, Rf 0.8; δ_H ($CDCl_3$,

80 MHz) 8.16 (4H, d, J_{AB} 8.8 Hz), 7.32 (4H, d, J_{AB} 8.8 Hz), 7.25-7.0 (5H, m, CH's on cinnamate), 4.62 (3H, m_{AB_2} $CHCH_2$-Bnpe), 2.7 (4H, $m_{A_2B_2}$, CH_2CH_2); 2-(4-nitrophenyl)-2(4-aminophenyl)ethyl dihydrocinnamate (*84*); t.l.c. - C, Rf 0.7, ninhydrin active; δ_H - spectrum obtained impure with diamino compound (*85*); 2,2-Bis(4-aminophenyl)ethyl dihydrocinnamate (*85*); t.l.c. - C, Rf 0.4, ninhydrin active; δ_H ($CDCl_3$, 80 MHz) 7.2 (5H, m, CH's cinnamate), 6.98 (4H, d, J_{AB} 8.4 Hz), 6.59 (4H, d, J_{AB} 8.4 Hz), 4.5 (2H, $m_{A_2B_2}$, CH_2-Bnpe), 4.2 (1H, m_{AB_2}, CH-Bnpe), 3.3 (4H, bs, 2-NH_2), 2.7 (4H, $m_{A_2B_2}$, CH_2CH_2); t.l.c. - G t = 25 min Rf 0.8 (no ninhydrin active spots); t = 40 min, Rf 0.7 (trace), Rf 0.8 (major).

(vii) <u>The action of five organic bases on a 0.3 molar solution of 2,2-Bis(4-nitrophenyl)ethyl acetate (*8*) in CD_2Cl_2</u>

0.5 ml (50 mg, 0.15 mmol) was withdrawn from a stock solution of (*8*) (0.3 g, 0.9 mmol) in CD_2Cl_2 (3 ml) and placed into a small vial. To this was added 1 equivalent of the required base, the solution homogenised and placed into a n.m.r. tube. Spectra were recorded at appropriate intervals to observe any formation of olefin (*28*), this having a characteristic peak at 5.8 ppm by ^1H n.m.r., well removed from other peaks.

(viii) The action of benzylamine on the acetates (*8*) and (*75*) was followed by the general H.P.L.C. method described in Section (3.6.1.).

2,2-Bis(4-nitrophenyl)ethyl acetate (*8*) (0.153 g, 0.46 mmol) was dissolved in dichloromethane (20 ml) to which was added benzylamine (50 µl, 0.46 mmol). The reaction was stirred for 2 hours and aliquots withdrawn to be analysed by H.P.L.C. This was repeated using 9-fluorenylmethyl acetate (*75*) (0.059 g, 0.46 mmol) in dichloromethane (10 ml) and benzylamine (26 µl, 0.25 mmol). H.P.L.C. chromatography showed a trace amount of olefin formation after forty minutes for (*8*) whilst up to 25% of the measured peaks represented olefin formation for FmocAc (*75*) over the same period.

Table 3.7

time	(*8*) Peak Area	Olefin Peak Area (% of total)	(*75*) Peak Area	Olefin Peak Area (% of total)
0	26000	-	13021	-
5	25518	-	21976	6966(25)
10	11683	<150	-	-
40	11174	<150	7652	2311(25)
120	-	-	7998	4323(35)

3.8 ELIMINATION STUDIES WITH 2,2-BIS(4-NITROPHENYL)-ETHYL CINNAMATE (*29*), DBN AND ACETIC ACID

For this series of experiments a standard solution of (*29*) (0.13 g, 0.3 mmol) in either dichloromethane (25 ml) or dimethylformamide (25 ml) (12 m.molar solution) was used. To this solution was added DBN (40 µl, 0.3 mmol) and the desired amount of acetic acid. Aliquots of 0.5 ml were withdrawn and worked-up in the standard manner (3.5.1). 15 µl was injected into the H.P.L.C. A:B (50:50) - B (100%) 25 min.

(i) (*29*) (0.14 g, 0.33 mmol), DBN (43 µl, 0.33 mmol) and acetic acid (19 µl, 0.33 mol) in dichloromethane (25 ml), slow/partial elimination. H.P.L.C. t = 0 RT = 18 min (21376); t = 40, RT = 5 min (acid), RT = 15 min (olefin), RT = 18 min (ester, 94% intact); t = 120 min, RT = 5 min (acid), RT = 15 min (olefin), RT = 18 min (ester, 83% intact).

(ii) Addition of DBN (43 µl, 0.33 mmol) to (i) produced complete elimination after 60 min. H.P.L.C. t = 5 min, RT = 5 min (12267), RT = 15 min (11434), RT = 18 min (12055); t = 65 min, RT = 5 min (17538), RT = 15 min (16647), RT = 18 min (758).

(iii) DBN (43 µl, 0.3 mmol), acetic acid (36 µl, 0.63 mmol) in DCM no elimination. H.P.L.C. t = 0, RT = 18 min; t = 120, RT = 18 min.

(iv) (*29*) (0.135 g, 0.31 mmol), DBN (40 µl, 0.3 mmol) and acetic acid (18 µl, 0.31 mmol) in DMF (25 ml). Elimination complete after 20 min. H.P.L.C. t = 0, RT = 18 min, t = 10 min, RT = 5 min (acid, 14811), RT = 15 min (olefin, 13698),

RT = 18 min (ester, 3618), t = 20 min, RT = 5 min (16476), RT = 15 min (15431), RT = 18 min (ester, 1087).

(v) DBN (39 µl, 0.3 mmol) and acetic acid (36 µl, 0.62 mmol) in DMF (25 ml). Elimination complete after 30-40 min. H.P.L.C. t = 0, RT = 18 min (ester, 23500); t = 10 min, RT = 5 min (11649), RT = 15 min (olefin, 10681), RT = 18 min (9703); t = 20 min, RT = 5 min (14394), RT = 15 min (13336), RT = 18 min (4817), t = 40 min, RT = 5 min (16039), RT = 15 min (35000).

(vi) DBN (40 µl, 0.33 mmol) and acetic acid (76 µl, 1.32 mmol) in DMF (25 ml). Elimination complete after about 60 minutes. H.P.L.C. t = 0, RT = 18 min (24750); t = 20 min, RT = 5 min (14190), RT = 15 min (13189), RT = 18 min (8839); t = 40 min, RT = 5 min (16329), RT = 15 min (15375), RT = 18 min (3963).

(vii) DBN (39 µl, 0.3 mmol) and acetic acid (144 µl, 2.5 mmol) in DMF (25 ml). Elimination in about 120 min. H.P.L.C. t = 40 min, RT = 5 min (9796), RT = 15 min (9251), RT = 18 min (12595); t = 120 min, RT = 5 min (14394), RT = 15 min (13689), RT = 18 min (2492).

(viii) DBN (40 µl, 0.3 mmol), trifluoroacetic acid (24 µl, 0.3 mmol) in DMF (25 ml). Limited elimination. H.P.L.C. t = 0, RT = 18 min (23475); t = 240 min, RT = 5 min (minor peak), RT = 15 min (minor peak), RT = 18 min (major peak).

(ix) Elimination of 2,2-Bis(4-nitrophenyl)ethyl cinnamate (*29*) with 20% piperidine/acetic acid

To a solution of (*29*) (0.10 g, 0.25 mmol) in dichloromethane (10 ml) was added acetic acid (1.16 ml, 20 mmol) and a sample removed for analysis by H.P.L.C. Piperidine (2 ml, 20 mmol) was then added thereby forming a 20% solution w/w in dichloromethane. Addition of the base was accompanied by an exotherm. Samples of 0.25 ml were taken at regular intervals and analysed as for 3.6.1. H.P.L.C., A:B (50:50) - B (100%) 25 min; t = 0, RT = 18 min (22000); t = 1 min, RT = 5 min (3878, acid), RT = 15 min (3429, olefin), RT = 18 min (7741), t = 10 min, RT = 5 min (7391, acid), RT = 15 min (4011, olefin), RT = 18 min (600, ester), t = 40 min, RT = 5 min (7691), RT = 15 min (3962), RT = 18 min (572). Elimination complete in 15-20 minutes.

(x) Elimination study with N-[2,2-Bis(4-nitrophenyl)-ethoxycarbonyl] valine (*56*) and DBN in dichloromethane

(A) (*56*) (0.137 g, 0.3 mmol), DBN (39 µl, 0.3 mmol) in DCM (25 ml). No elimination. H.P.L.C. t = 0, RT = 9 min; t = 100, RT = 9 min.

(B) DBN (39 µl, 0.3 mmol) was added to (A). Complete elimination was obtained after 60 minutes. H.P.L.C. t = 60 min, RT = 9 min (1057) RT = 15 min (15227). (c.f. 3.8(ii)).

3.8.1 Elimination of N-[9-fluorenylmethoxycarbonyl]-alanine benzylamide (79) with DBN (acetic acid)

(i) To a solution of (79) (35.6 mg, 0.09 mmol) in dimethylformamide (7 ml) (12.5 mmolar soln.) was added DBN (11 µl, 0.09 mmol) turning the initially pale yellow solution clear. Samples of 0.5 ml were withdrawn at regular intervals and analysed by H.P.L.C. as in 3.6.1.

H.P.L.C. A:B (50:50) - B (100%) 25 min; t = 0, RT = 12 min (18648, amide); t = 30 seconds, 5 ml sample of reaction fluid. RT = 12 min (8407), RT = 17 min (24350, olefin, 73% of total peak area); t = 10 min, RT = 17 min (11369, olefin), elimination complete less than 10 minutes.

(ii) This procedure was repeated using (79) (33.5 mg, 0.084 mmol) in dimethylformamide (7 ml), DBN (11 µl, 0.09 mmol) and acetic acid (10 µl, 0.18 mmol, 2 equivs.). Samples were taken of 0.5 ml and analysed as for (i).
H.P.L.C. A:B (50:50) - B (100%), 25 min; t = 0, RT = 12 min (16746); t = 10 min, RT = 12 min (8749, amide), RT = 17 min (827, olefin), t = 20 min, RT = 12 min (925, amide), RT = 17 min (5194, olefin); t = 45 min, RT = 12 min (13559), elimination complete between 20-45 min.

3.8.2 Elimination studies using 7.35 mmolar solutions of N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]alanyl phenylalanylglycine methyl ester (70)

(i) To (70) (0.091 g, 0.15 mmol) in dichloromethane (20 ml)

was added DBN (18.5 µl, 0.15 mmol) at 11°C. Samples of 0.3 ml were withdrawn at appropriate intervals, worked-up as described in the general method of 3.6.1, and 15 µl aliquots injected into the H.P.L.C. Complete elimination observed after 3 hours. H.P.L.C. A:B (50:50) - B (100%) 25 minutes, (*70*) RT = 10 min; olefin (*28*), RT = 15 mins.

(ii) The experiment was repeated using (*7C*) (0.090 g, 0.15 mmol) in dichloromethane (20 ml) with DBN (36 µl, 0.3 mmol, 2 equiv.). Complete disappearance of (*70*) peak at RT = 10 min, replaced by the olefin (*28*) at RT = 15 min was recorded after eighty-four minutes.

(iii) To (*70*) (0.092 g,0.15 mmol) in dimethylformamide was added DBN (18.4 µl,0.15 mmol). Aliquots of 0.3 ml were withdrawn and analysed by H.P.L.C. to record the disappearance of the (*70*) peak. Complete elimination was recorded after 35 minutes. H.P.L.C. A:B (50:50) - B (100%), t = 1 min 40s, RT = 10 min (7889), RT = 14 min (1050); t = 15 min, RT = 10 min (2730); RT = 14 min (5195), t = 25 min, RT = 10 min (649), RT = 15 min (7057); t = 37 min, RT = 15 min (7688).

(iv) To (*70*) (0.096 g, 0.155 mmol) in dimethylformamide (20 ml) at 18°C was added DBU (23.5 µl, 0.155 mmol). Aliquots of 0.5 ml were withdrawn, worked-up and analysed as in 3.6.1 by H.P.L.C. This showed complete elimination after 22 minutes. H.P.L.C. A:B (50:50) - B (100%) 25 min, t = 3 min 35s. RT = 10 min, RT = 11 min, RT = 14 min

(olefin), t = 22 min, RT = 14 min (olefin).

(v) To (*70*) (0.062 g, 0.1 mmol) in dimethylformamide (10 ml) was added acetic acid (6 µl, 0.1 mmol) and DBN (12.5 µl, 0.1 mmol). Aliquots of 0.5 ml were withdrawn, worked-up and analysed as for 3.6.1. Complete elimination was recorded after 30 minutes (c.f. iii). H.P.L.C. A:B (50:50) - B (100%) 25 min, t = 1 min 30s. RT = 11 min (urethane) (major peak), RT = 14 min (olefin, minor peak), t = 25 min, RT = 11 min (minor peak), RT = 14 min (major peak), t = 35 min, RT = 14 min.

(vi) Stability study of Bnpeoc Ala Phe Gly OMe (*70*) to sodium hydroxide solution

To (*70*) (0.051 g, 0.083 mmol) in dimethylformamide (3 ml) was added 0.025M NaOH (0.083 mmol). The initially clear solution formed a precipitate with considerable evolution of heat. This was allowed to stir for ninety minutes before the reaction was poured into water (50 ml), acidified with conc.HCl to pH 2 and extracted with ethyl acetate. The combined organic phase was washed with water (15 ml x 2), brine, dried over Na_2SO_4 and reduced *in vacuo* to produce a foam (0.058 g), t.l.c. - D, Rf 0.2 (acid (*80*)) Rf 0.62 (ester (*70*)), Rf 0.9 (v.faint, olefin), (no ninhydrin active spots).

This experiment was repeated with (*70*) (0.44 g, 0.7 mmol) in dimethylformamide (10 ml) and 0.05M NaOH (14.3 ml, 28 mmol). On addition of the base a blue colour formed, with a large evolution of heat which turned clear leaving

a precipitate. The reaction was worked-up after twenty-five minutes and the products separated by chromatography on silica ($CHCl_3$). The products obtained from the column provided the N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]alanyl phenylalanylglycine (*80*), 60%, olefin as 5% and starting material as the remaining 35%.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl)alanylphenylalanyl-glycine (*80*)

T.l.c. - D, Rf 0.2, H.P.L.C. A:B (50:50) - B (100%) 25 min; RT = 8 min; RT (olefin) = 16 min; δ_H [$(CD_3)_2SO$, 200 MHz] 8.18 (4H, d, J_{AB} 8.6 Hz), 8.06 (1H, m, NH urethane) 7.85 (1H, m, NH Gly), 7.67 (4H, d, J_{AB} 8.5 Hz), 7.31 (1H, m, NH), 7.2 (5H, m, CH x 5 Phe), 4.64 (4H, m, CH,CH_2 Bnpeoc, α CH Phe), 4.0 (1H, m, α CH.Ala), 3.6 (bs, DMSO), 2.9 (2H, m_{ABX}, CH_2.Gly), 1.1 (3H, d, J 6.7 Hz); m/z (FAB) - nominal mass, 609 (m+1), 579, 564, 534, 506, 469, 241, 223, 120. Amino Acid Analysis: Gly:Ala:Phe; 1.07:0.93:0.99.

(vii) Elimination study with 1,5,7-triazabicyclo[4.4.0]dec-5-ene resin (3.2 mmol/g); TBD - resin (*126*)

(A) TBD-resin (0.13 g, 0.32 mmol) was added to a solution of Bnpe-cinnamate (*29*) (0.13 g, 0.3 mmol) in dichloromethane (25 ml). Samples of 0.5 ml were withdrawn at appropriate intervals and filtered through a cotton-wool bud in a pasteur pipette. The residue obtained after removal of the solvent *in vacuo* from the filtrate was dissolved in acetonitrile (2 ml) from which was injected 15 μl into the H.P.L.C. The resin was filtered off after 24 hours and

the solvent removed *in vacuo* from the filtrate to produce a residue which, by H.P.L.C., consisted mainly of olefin (*28*) with some ester (*29*). The resin was then washed thoroughly with an acetic acid/water (9:1) solution which on removal of the aqueous phase by freeze-drying produced a yellow solid (21 mg). A further amount of this product was isolated from acidification of a base wash of the organic phase. Total weight obtained (27 mg). H.P.L.C. A:B (50:50) - B (100%) 25 min, RT = 5 min, cinnamic acid standard RT = 5 min, m.p. = 128-131°C (lit., 133-134°C). Yield for recovery of cinnamic acid, 70%. H.P.L.C. A:B (50:50) - B (100%) 25 min, time course t = 0, RT = 19 min (26313); t = 180 min, RT = 5 min (2473, acid), RT = 16 min (5331, olefin), RT = 19 min (18725, ester); t = 24 hours, RT = 5 min (1393, acid), RT = 16 min (10740, olefin), RT = 19 min (4088, ester). (The acid peak is artificially small, due to binding to base); ν_{max} (CH_2Cl_2), cinnamic acid from resin, 3000(b), 1680 (CO) and 1640 cm^{-1} (olefin).

This experiment was repeated with Bnpe cinnamate (0.35 mmol) dichloromethane (12 ml) and TBD-resin (10 equivs); H.P.L.C. A:B (50:50) - B (100%), t = 5 min, RT = 5 min (7293, acid), RT = 16 min (7000, olefin), RT = 19 min (14527, ester); t = 25 min, RT = 5 min (14790, acid), RT = 16 min (12250, olefin), RT = 19 min (186, ester). Elimination complete after 30 min. The resin was filtered off and the solvent removed to produce a residue shown by H.P.L.C. to contain no ester.

(B) To Bnpeoc Ala Phe Gly OMe (*70*) (0.2 g, 0.32 mmol) in dimethylformamide (25 ml) was added TBD - resin (0.13 g, 1.3 eq.), 5 ml of solvent used to rinse out reagent vial. Aliquots of 0.2 ml were withdrawn and analysed by H.P.L.C. as described for (i) for 8 hours after which stirring was continued for twenty-four hours before the resin was filtered off and washed with aqueous dimethyl/ formamide. The solvent was removed under reduced pressure to produce a white solid (0.32 g), crystallised from methanol to give pure olefin (*28*) (0.06 g) as shown by H.P.L.C. The filtrate was reduced *in vacuo* to produce a residue (80 mg) shown by t.l.c. to be a mixture of olefin (*28*) and a polar ninhydrin active material. The remaining resin was washed with acetic acid/water (9:1), filtered from which the aqueous phase was removed by freeze drying to produce a gum (50 mg) crystallised from chloroform.
H.P.L.C. A:B (50:50) - B (100%) 25 min, t = 0, RT = 11 min, t = 4 hrs, RT = 16 min (3718, olefin), RT = 11 min (3952), t = 8 hrs, RT = 11 min (1.0); RT = 16 min (4.5).
Amino acid analysis of residue Gly:Ala:Phe; 1.03:0.90:1.07.

3.9 SOLID PHASE PEPTIDE CHEMISTRY

3.9.1 Preparation of Bnpeoc-L-valine symmetrical anhydride (*95*)

To a solution of Bnpeoc-L-valine (*95*) (0.262 g, 0.6 mmol) in dichloromethane (10 ml) at 0°C was added dicyclohexylcarbodiimide (62 mg, 0.3 mmol) in dichloromethane (2 ml). The mixture was stirred for ten minutes before the precipitated urea was removed by filtration and the filtrate evaporated to dryness. The resulting foam was dissolved in dichloromethane (2 ml) and used directly.

Preparation of Bnpeoc-L-valine acid chloride (*93*)

A solution of Bnpeoc valine (0.73 g, 1.7 mmol) in dichloromethane (25 ml) was treated with thionyl chloride (1.2 ml, 1.7 mmol) and the mixture refluxed for 1 hour under argon. Solvent and excess thionyl chloride was removed under reduced pressure, the residue redissolved in dichloromethane and again reduced *in vacuo* to produce a gum. The procedure was repeated a further two times to ensure complete removal of excess thionyl chloride. The residue was then dissolved in the required amount of either dichloromethane or dimethylformamide solvent and used immediately, ν_{max} (CH_2Cl_2) 3450 (NH), 1805 (CO, acid chloride), 1740 (CO, urethane), 1610, 1600 (C-C, aromatic), 1520, 1350 (NO_2) cm^{-1}. Bnpeoc glycine acid chloride (*94*) was prepared in a similar fashion other than the initial dichloromethane suspension only dissolved at reflux.

(i) N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-L-valine p-alkoxybenzyl ester (styrene 1% divinylbenzene) resin (Bnpeoc Val-OCH_2-C_6H_4-OCH, C_6H_4-resin (*127*)

To p-alkoxybenzyl alcohol resin (0.22 g, 0.15 mmol) swollen in dichloromethane (10 ml) was added a solution of preformed Bnpeoc valine symmetrical anhydride (0.6 ml) in dichloromethane (2 ml) and 4-dimethylaminopyridine (0.03 mmol, 0.36 ml of a 10% solution in DCM). The mixture was stirred for 2 hours at room temperature after which the resin was filtered and washed thoroughly with dichloromethane. The resin was dried and analysed by i.r. and CHN. ν_{max} 3580 (OH), 3425 (NH), 1740 (CO, urethane and ester), 1520, 1350 cm^{-1} (NO_2), (Found: N, 1.18; $C_{20}H_{19}N_3O_7$-resin requires: N, 2.21); esterification = 47%.

(ii) Investigation into the reactivity of Bnpeoc Val-Cl (*93*) with p-alkoxybenzyl alcohol resin

For this series of experiments four mixtures of p-alkoxybenzyl alcohol resin (0.1 g, 0.064 mmol) swollen in a minimum volume of dichloromethane (3 ml) under argon were treated as indicated below with N-methylmorpholine and/or 4-dimethylaminopyridine (DMAP). To these stirred suspensions was added Bnpeoc Val-Cl (0.26 mmol) dissolved in dichloromethane (1 ml). The resulting mixtures were stirred for one hour after which the resin was filtered off and thoroughly washed with dichloromethane. I.r. spectra for the resin and filtrate were obtained, the former analysed by CHN also.

(a) N-Methyl morpholine (56 µl, 0.12 mmol), ν_{max} (KBr) 3450 (b s, OH), no ester peak; ν_{max} (CH_2Cl_2) 2900 (CH), 2500-2000 (salt), 1840 (CO, oxazolone), 1730 (CO, urethane), 1680 (CO, oxazalone), 1520 and 1350 cm^{-1} (NO_2).

(b) N-Methyl morpholine (56 ml, 0.12 mmol), DMAP (0.7 mg, 0.6 µmol, 1% mole/mole acid). Only small amount esterified as shown by i.r. ν_{max} (KBr) 3580 (OH), 3470 (NH), 1740 (CO, ester, urethane), 1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2) 2900 (CH), 2500-2000 (salt) 1840, 1680 (CO, oxazalone), 1730(w), (CO), 1520 and 1350 cm^{-1} (NO_2).

(c) N-Methyl morpholine (56 ml, 0.12 mmol), DMAP (1.6 mg, 16.4 µmol, 5% mole/mole acid chloride), esterification increased; ν_{max} (KBr) 3580 (OH); 3440 (NH), 1735 (CO, ester and urethane), 1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2) 2980 (C-H) 2500-2000 (salt), 1730 (CO, urethane), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 1.06%; $C_{20}H_{19}N_3O_7$-resin requires: N, 2.17), esterification = 49%.

(d) 2,6-Lutidine (0.18 ml, 1.53 mmol); DMAP (1.6 mg, 16.4 µmol, 5% mole/mole acid chloride), ν_{max} (KBr) 3580 (OH), 3440 (NH), 17.35 (CO, ester and urethane), 1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2) 2980 (CH), 2500-2000 (salt), 1730 (CO, urethane), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 0.68; $C_{20}H_{19}N_3O_7$-resin requires: N, 2.17); esterification = 31%.

3.9.2 N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]valine p-alkoxybenzyl ester resin (*96*)

(i) Prepared using manual shaker with NMM/5% DMAP/Bnpeoc Val-Cl

To p-alkoxybenzyl alcohol resin (0.5 g, 0.33 mmol) swollen in dichloromethane (20 ml) was added N-methyl morpholine (55 µl, 0.5 mmol), DMAP (25 mmol in 0.3 ml. DCM) and finally Bnpeoc Val-Cl (0.5 mmol) in dichloromethane (1 ml). This mixture was shaken for 30 min after which the solvent was removed and the resin washed thoroughly with dichloromethane and dimethylformamide. A resin sample was retained for i.r. and CHN analysis. The remaining resin was reswollen in dimethylformamide (20 ml) to which was added N-methylmorpholine (74 µl, 0.67 mmol), DMAP (33.5 µl in 0.41 ml DCM) and finally Bnpeoc Val-Cl (0.67 mmol) in dichloromethane (1 ml). This mixture was allowed to shake for 30 min after which the resin was filtered, washed and dried. Results obtained for esterification with dichloromethane solvent; ν_{max} (KBr) 3600 (OH), 3480 (NH), 1740 (CO, ester, urethane) 1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2) 2500-2000 (salt), 1840, 1680 (oxazalone) 1520 and 1350 cm^{-1} (NO_2); (Found: N, 0.48; $C_{20}H_{19}N_3O_7$-resin, N, 2.2%); esterification = 18%. Results obtained for esterification with DMF as solvent, ν_{max} (KBr) 3600 (OH), 3480 (NH), 1740 (CO, ester, urethane) 1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2), 2500-2000 (salt), 1740 (CO, urethane), 1680 (oxazalone), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 0.58; $C_{20}H_{19}N_3O_7$ requires: N, 2.2%), esterification = 22%.

(ii) N-[2,2-Bis-(4-nitrophenyl)ethoxycarbonyl]-L-valine p-alkoxybenzyl ester resin (*98*)

Prepared using sonic bath with NMM, 5% DMAP, Bnpeoc Val-Cl

To p-alkoxybenzyl alcohol resin (0.5 g, 0.335 mmol) in dichloromethane (sufficient to obtain a 'mobile slurry') (10 ml) was added N-methylmorpholine (55.2 µl, 0.5 mmol) and DMAP (25 µmol in 0.3 ml DCM). This mixture under argon was treated with Bnpeoc Val-Cl (0.5 mmol) in dichloromethane (1.5 ml) and placed into a sonic bath for 1 hour. The resin was then filtered and washed thoroughly with dichloromethane, a sample of resin and filtrate was retained for i.r. and CHN analysis. The remaining resin was reswollen in dimethylformamide (10 ml) to which was added N-methylmorpholine (0.335 mmol), DMAP (25 µmol) and Bnpeoc Val-Cl (0.335 mmol). This mixture was allowed to stand in a sonic bath for 1 hour after which the resin was filtered and washed as before.

Results obtained for esterification with dichloromethane as solvent.

ν_{max} (KBr) 3600 (OH), 3480 (NH), 1740 (CO, urethane, ester), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 1.1; $C_{20}H_{19}N_3O_7$-resin requires: N, 2.2), esterification = 40%; ν_{max} (CH_2Cl_2) 2500-2000 (salt), 1740 (CO, urethane), 1520 and 1350 cm^{-1} (NO_2).

Results obtained for esterification with dimethylformamide as solvent

ν_{max} (KBr) 3600 (OH), 3480 (NH), 1740 (CO, urethane, ester), 1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2) 2500-2000 (salt), 1740 (CO, urethane), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 1.27; $C_{20}H_{19}N_3O_7$-resin requires: 2.2%); esterification = 47%.

3.9.3 N-[2,2-Bis-(4-nitrophenyl)ethoxycarbonyl]glycine p-alkoxybenzyl ester copolymer (styrene, 1% divinyl benzene) resin (*97*)

Prepared using manual shaker, NMM, 5% DMAP, Bnpeoc Gly-Cl

p-Alkoxybenzyl alcohol resin (1.07 g, 0.67 mmol) in dichloromethane (20 ml) was treated with N-methylmorpholine (110.5 µl, 1 mmol), DMAP (5 mg, 51 µmol, 5% mol/mol) and Bnpeoc Gly-Cl (1 mmol) and shaken for 1 hour. After which time the resin was filtered, washed and dried (a sample was taken for i.r. and CHN analysis). To the remaining resin swollen in dimethylformamide (20 ml) was added N-methylmorpholine (74 µl, 0.67 mmol in 1 ml DCM). This mixture was shaken for thirty minutes before the solvent was removed and the resin thoroughly washed with dimethylformamide (x3) and dichloromethane (x3). The resin was dried to give (**97**) (1.2 g).

Results obtained for esterification with dichloromethane as solvent

ν_{max} (KBr) 3600 (OH), 3450 (NH), 1745 (urethane, ester),

1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2) 2500-2000 (salt), 1740 (urethane), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 1.12; $C_{17}H_{13}N_3O_7$-resin requires: N, 2.2%), esterification = 44%.

Results obtained for esterification with dimethylformamide

ν_{max} (KBr) 3440 (NH), 1740 (CO, urethane, ester), 1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2) 2500-2000 (salt), 1740 (urethane), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 1.90; $C_{17}H_{13}N_3O_7$-resin requires: N, 2.2%); esterification = 81%. The above resin was capped with acetic anhydride/triethylamine and deprotected with DBU/acetic acid as described for (*104*).

3.9.4 General method used for capping and deprotection of Bnpeoc A.A-resins

Valine p-alkoxybenzyl ester copolymer (styrene, 1% divinyl benzene) resin

Bnpeoc valine p-alkoxybenzyl ester resin (1.3 g, 0.34 mmol) swollen in dichloromethane (20 ml) was shaken with acetic anhydride (0.25 ml, 2.6 mmol) and triethylamine (0.36 ml, 2.6 mmol) for one hour. The solvent was removed and the resin washed with dichloromethane before the above procedure was repeated with dimethylformamide as solvent. The resin was dried and a sample was taken for i.r. ν_{max} (KBr) 3450 (NH), 1740 (CO, ester, urethane), 1520 and 1350 cm^{-1} (NO_2). The above resin was swollen in dimethylformamide (20 ml) and acetic acid was added (0.12 ml, 0.8 mmol) and 1,8-diazabicyclo[4.3.0]undec-8-ene (DBU) (0.8 mmol). The mixture was

shaken for ten minutes before the solvent was drained off and the resin washed with dichloromethane (twice) and dimethylformamide (twice). This procedure was repeated with another amount of DBU/acetic acid for ten minutes after which the resin was washed as above with an additional 15 min wash with dichloromethane. The solvent was removed and the resin dried to provide (1.3 g), ν_{max} (KBr) 1740 cm^{-1} (ester, acetate).

3.9.5 General method for GlyGly analysis

Glycine p-alkoxybenzyl ester resin (52 mg, 23 mmol) was treated with a dichloromethane/trifluoroacetic acid (3 ml) mixture (1:1) for 1 hour in a sonic bath. The resin was filtered off and washed thoroughly with dichloromethane removing the pink colouration from the resin. The residue produced on removal of the solvent *in vacuo* was dissolved in 0.2M sodium citrate buffer (100 ml), pH 3.49, from which was placed 120 µl onto an ion-exchange column for amino acid analysis. Table 2.21 summarises the results obtained.

3.9.6 Study into the prevention of Gly-Gly dimer formation on resin

1 Bnpeoc-Gly-resin using sonic bath with NMM/5% DMAP/ Bnpeoc-Gly-Cl

(i) p-Alkoxybenzyl alcohol resin (0.206 g, 0.14 mmol) was swollen in minimal volume of dichloromethane (1ml) to cause formation of 'mobile slurry'. To this was added N-methyl morpholine (15.4 µl, 0.14 mmol), DMAP (6.9 µmol), and Bnpeoc Gly-Cl (0.14 mmol in 1 ml DCM). This was allowed to stand in a sonic bath for 1 hour after which the resin was filtered off, washed thoroughly with dichloromethane and dried. The resin (*100*) and filtrate were analysed by i.r. (the former by CHN also); ν_{max} (KBr) 3580 (OH), 3440 (NH), 1735 (CO, urethane and ester), 1520 and 1350 cm^{-1} (NO_2);

ν_{max} (CH_2Cl_2) 2500-2000 (salt) 1735 (CO, urethane), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 0.59; $C_{17}H_{13}N_3O_7$-resin requires: N, 2.25%); esterification = 22%.

(ii) 1 Bnpeoc Gly-resin prepared using sonic bath/pyridine/ Bnpeoc Gly-Cl

To p-alkoxybenzyl resin (0.206 g, 0.14 mmol) was added sufficient dichloromethane (1 ml) to dry swell the resin, sufficient pyridine (2 ml) to obtain a slurry and Bnpeoc Gly-Cl (0.14 mmol in 1 ml DCM). This mixture was allowed to stand in a sonic bath for 90 minutes after which the resin was filtered off and washed thoroughly with dichloromethane (5 x 5 ml). The resin (*101*) and filtrate were analysed by i.r. and CHN as before. ν_{max} (KBr) 3420 (NH), 1740 (CO, urethane, ester), 1520 and 1350 cm^{-1} (NO_2); ν_{max} (CH_2Cl_2) 2500-1950 (pyridinium salt), 1720 (CO, urethane), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 2.04; $C_{17}H_{13}N_3O_7$-resin requires: N, 2.25); esterification = 88%.

3.9.7 N-[2,2-Bis-(4-nitrophenyl)ethoxycarbonyl]-L-valine p-alkoxy benzyl ester resin (*103*)

Use of 1 Bnpeoc Val-Cl in pyridine

(i) To p-alkoxybenzyl alcohol resin (0.93 g, 0.94 mmol) was added dichloromethane (2 ml), pyridine (4 ml) and Bnpeoc Val-Cl (*93*) (0.94 mmol in 1.5 ml DCM). The mixture was allowed to stand in a sonic bath for 2 hours and worked-up as for Bnpeoc Gly-resin (*100*). ν_{max} (KBr) 3570 (OH), 3454 (NH), 1730 (CO, urethane, ester), 1520 and 1350 cm^{-1}

(NO_2); (Found: N, 2.06; $C_{20}H_{19}N_3O_7$-resin requires: N, 3.0%); esterification = 60%.

(ii) Use of 1.1 Bnpeoc Val-Cl in pyridine

The above experiment (i) was repeated with p-alkoxybenzyl alcohol resin (1.49 g, 1.51 mmol) in dichloromethane (3 ml) and pyridine (6 ml) to which was added Bnpeoc Val-Cl (1.65 mmol, 1.1 eq) in 2 ml dichloromethane. Work-up after 2 hours in a sonic bath was as described in (i) (Bnpeoc Gly-Cl) to give (*104*) (1.8 g); ν_{max} (KBr) 3420 (NH), 1730 (CO, urethane, ester), 1520 and 1350 cm^{-1} (NO_2); (Found: N, 2.45; $C_{20}H_{19}N_3O_7$-resin requires: N, 3.0%); esterification = 75%.

(iii) Racemisation study with valine-p-alkoxybenzyl ester resin (*98*)

To (*98*) (0.209 g, 84 mmol) in dichloromethane (2.5 ml) was added t-butyloxycarbonylalanine (49 mg, 0.25 mmol) in dichloromethane (2 ml) and dicyclohexylcarbodiimide (54 mg, 0.25 mmol). The mixture was stirred under argon for 2 hours after which the resin was washed thoroughly with dichloromethane. The resin was treated with a trifluoroacetic acid/dichloromethane mixture (5 ml, 1:1) for 1 hour in a sonic bath. The resin was then filtered off and washed thoroughly. The residue obtained on removal of the solvent from the filtrate was dissolved in 100 ml 0.2M sodium citrate buffer (pH 3.49), and from which was taken 120 ml and placed on an ion-exchange column at 75°C for amino acid analysis.

(iv) Racemisation study on Bnpeoc Val-resin (*104*)

Bnpeoc Val-resin (*104*) (1.8 g, 1.5 mmol) was swollen in dichloromethane (4 ml) and pyridine (10 ml) to which was added acetic anhydride (0.71 ml, 5 mmol). The resin was filtered and washed with dichloromethane after 1 hour in a sonic bath. Treatment with a solution of DBU/acetic acid in dimethylacetamide was as described in Section 3.9.4 to produce valine benzyl ester resin (*105*).

To (*105*) (0.11 g, 67 μmol) in dichloromethane (3 ml) was added ^{t}-Butyloxycarbonylalanine (38 mg, 0.2 mmol) and dicyclohexylcarbodiimide (41.5 mg, 0.2 mmol). Work-up of the reaction was as described in (iii). The residue from the cleaved filtrate was dissolved in pH 3.49 buffer ; from which 120 μl was placed onto an ion-exchange amino acid analyser.

3.9.8 Solid phase peptide synthesis

(i) N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]phenylalanine leucylalanylglycine benzylamide copolymer (styrene, 1% divinylbenzene) (*109*)

Bnpeoc Phe-Leu-Ala-Gly-resin (*109*)

The resin bound peptide (*109*) was prepared using the following solid phase cycle: (1) DCM, 2 x 5 min, (2) coupling Bnpeoc Gly-ODpp mixed anhydride (1.5 equivalents), 1 x 30 min, Bnpeoc Gly-ODpp mixed anhydride (10 equivalent), 1 x 30 min, (3) DCM, 2 x 5 min, (4) DMF, 2 x 5 min, (5) DBN/AcOH (1 equivalent), DMF 2 x 10 min, (deprotection study at this step); (6) DCM, 2 x 5 min, (7) DMF, 2 x 5 min, (8) Bnpeoc

Ala-ODpp mixed anhydride (2 equivs), 1 x 30 min, (9) DBN/HOAc (2 equivs), DMF, 1 x 15 min, (10) Bnpeoc Leu-ODpp mixed anhydride (2 equivs), DCM, 2 x 30 min, (11) DBN/HOAc (1.2 equivs), DMF 2 x 30 min (deprotection study at this stage), (12) DCM, 2 x 5 min, (13) DMF, 2 x 5 min, (14) Bnpeoc Phe ODpp mixed anhydride, 3 x 30 min, (15) Ac_2O/pyridine, 1 x 30 min, (16) DMF, 2 x 5 min, (17) DCM, 2 x 5 min. Samples of resin after coupling steps (2), (8), (10) and (14) were taken for Kaiser test, if a positive result (blue) was obtained then a further equivalent of acylating reagent was added. Only for step (8) with Bnpeoc Ala to Glycyl-resin was a recoupling step not required, whilst a negative Kaiser test was not obtained for the final coupling (step (14)). On addition of the Bnpeoc A.A mixed anhydride an equivalent of 2,6-lutidine was added as in the general procedure. After the final step (17) the resin was filtered off and dried. A sample was taken for hydrolysis and analysis by amino acid analysis. Amino Acid Analysis of crude peptide (*109*) is given in discussion Section 2.7.

(ii) N-[Benzyloxycarbonyl]leucylalanylglycylvaline (*110*)

Z-Leu-Ala-Gly-Val-OH (*110*)

The peptide (*110*) was prepared on p-alkoxybenzylalcohol resin (0.5 mmol) using an Applied Biosystems Automated Peptide Synthesiser. The following cycle was used.

HO-resin loaded (0.5 mmol, 0.67 mmol/g), (1) DCM, 2 x 10 min; DMA 2 x 10 min, (2) Bnpeoc Val anhydride, from Bnpeoc Val-OH, Diisopropylcarbodiimide (1 mmol), DMA, 2 x 30

min with catalytic amount DMAP, (3) drain, wash, DMA 8 x 36 seconds, (4) DBU/HOAc (1.2 eq) 180 seconds, (5) drain, (6) DBU/HOAc (1.2 eq) 425 seconds, (6) drain, wash, DMA, 11 x 36 seconds, (7) Bnpeoc Gly-OH (1 mmol) diisopropylcarbodiimide/HOBt 2 x 1800 seconds, (8) drain, wash cycle (3), (9) DBU/HOAc (1.2 eq) 180 seconds, (10) drain, (11) DBU/HOAc (1.2 eq) 425 seconds, (12) wash cycle (6), (12) Bnpeoc Ala-OH, diisopropylcarbodimide/ HOBt (1 mmol) 2 x 30 min, (13) wash cycle (3), (14) DBU/ HOAc (1.2 eq) 180 seconds, (15) drain, (16) DBU/HOAc (1.2 eq) 425 seconds, (17) drain, (18) wash cycle (6), (19) benzyloxycarbonyl leucine, diisopropylcarbodiimide/HOBt (1 mmol) 2 x 30 min, (20) wash cycle (3), (21) drain.

The peptide was cleaved from the resin with a trifluoro-acetic acid/dichloromethane (1:1) mixture. The resin was filtered off and the filtrate reduced *in vacuo* to produce a clear glass (0.2 g). A 50 mg portion of this material was dissolved in methanol (5 ml) to which was added 10% Pd/C (5 mg) and stirred over a hydrogen atmosphere for 18 hours. Removal of the catalyst produced a clear liquid reduced *in vacuo*. The residue was dissolved in 0.2M sodium citrate buffer (pH 3.49) from which 120 µl was applied to an ion-exchange column of an amino acid analyser at 75°C. Appendix (C), spectrum (12). Samples from the crude peptide *(110)* were taken for acid analysis (Table (38), page 189). m/z (FAB) L.R.M.S. 852, 808, 614, 585, 515, 493, 477; H.R.M.S. for peak at 493; Found: 493.26618; $C_{24}H_{37}N_4O_7$ requires: 493.26620 (.˙. =<1 ppm).

(iii) N-[Benzyloxycarbonyl]leucylalanylglycylvaline (*111*)

Z-Leu-Ala-Gly-Val-OH (*111*)

This peptide was prepared on p-alkoxybenzyl resin (0.5 mmol, 1.01 mmol/g) as for peptide (*110*) except that step (2) was removed and Bnpeoc Val p-alkoxybenzyl ester resin (*104*) used in its place. Step (7) was performed with Bnpeoc Gly-OH 1 mmol, total, i.e. no double coupling. This change led to a poor coupling (85% as judged by Kaiser test), the resin was capped with acetic anhydride/triethylamine after step (12).

Cleavage of the peptide from the resin was achieved as for (*110*). Samples were taken for amino acid analysis (Table (3.8) page *189*) and purity analysis (Appendix C, spectrum 12). The crude material (*111*) was then crystallised from chloroform to produce a white solid from which samples were taken for purity analysis (Appendix (C), spectrum (B)) and amino acid analysis (Table 3.8, page *189*).

m/z (FAB) For crystallised peptide (*112*); Found: 493.26618; $C_{24}H_{37}N_4O_7$ requires 493.26618 (.˙.=<1 ppm).

3.9.9 Deprotection study on Bnpeoc A.A.-resin

(i) N-[2,2-Bis-(4-nitrophenyl)ethoxycarbonyl]-leucylalanyl glycine amide methyl resin Bnpeoc Leu-Ala-Gly-NH-Resin

(*108*) constructed from acylation of the amino resin with 2 equivalents of Bnpeoc amino acid diphenylphosphinyl mixed anhydride after the required removal of Bnpeoc groups.

Determination of Optimum Deprotection Time using 1.2 Equivalents DBN/acetic Acid

To (*108*) (0.15 mmol) in dimethylformamide (15 ml) was added acetic acid (13 µl, 0.18 mmol) and DBN (23.4 µl, 0.18 mmol) and the mixture shaken. After the required shaking time an aliquot of 50 ml was withdrawn, from which was taken 10 µl and injected into the H.P.L.C. From this was obtained chromatograms of olefin production. After a total contact time of 35 minutes the resin was filtered off and washed thoroughly with dichloromethane and dimethylformamide. The resin was reswollen in dimethylformamide (15 ml) and treated with fresh amounts of DBN for 10 minutes shaking after which a chromatogram was obtained as described above. H.P.L.C. A:B (1:4) isocratic t = 5 min, RT = 4 min (olefin, ratio of internal standard: olefin peak = 4.8); t = 10 min, R = 4 min (5.0); t = 35 min, RT = 4 min (5.3); Second, DBN/acetic acid wash t = 10 min, no olefin.

(ii) N-[2,2-Bis-(4-nitrophenyl)ethoxycarbonyl]-L-valine p-alkoxybenzyl ester resin (*104*) with DBU/acetic acid/dimethylacetamide/sonic bath

Bnpeoc Val-resin(*104*) (0.152 g, 0.18 mmol) swollen in dimethyl acetamide (1.8 ml, 0.20 scale of machine prep) was treated with a 0.3M solution of DBU/acetic acid in dimethylacetamide (0.4 ml, 1.2 eq, as used in automated run) and placed into a sonic bath. Immediately on addition a stop watch was started and aliquots of 50 ml were withdrawn at appropriate intervals. These samples were filtered through a cotton wool bud in a pasteur pipette and washed with dichloromethane (5 ml). The combined filtrate was reduced *in vacuo* and the residue dissolved in acetonitrile (1 ml) from which was injected 15 µl into the H.P.L.C. After 30 minutes reaction the resin was filtered off and washed thoroughly with dichloromethane. The dry resin was reswollen in dimethylacetamide (1.8 ml) to which was added a fresh solution of DBU/acetic acid in dimethylacetamide (0.4 ml, 1.2 eq). This was left to stand in a sonic bath for 25 minutes after which a sample was taken as described above.

H.P.L.C., A:B (1:4) isocratic t = 6 min, RT = 5 min (7139); t = 12 min, RT = 5 min (6510); t = 30 min, RT = 5 min (7978) second DBU/acetic acid/DMA wash; t = 25 min, no olefin.

3.10 PHOSPHORUS DERIVATIVES

2,2-Bis-(4-nitrophenyl)ethyl phosphorus dichloridite (*116*)

To a solution of PCl_3 (0.53 ml, 6.05 mmol) in 2 ml acetonitrile over nitrogen was added 2,2-Bis-(4-nitrophenyl)-ethanol (*1*) (0.25 g, 0.86 mmol) in one portion. After

stirring for ten minutes the solvent was removed *in vacuo* to produce a crystalline solid. δ_H ($CDCl_3$, 80 MHz) 8.22 (4H, d, J_{AB} 8.8 Hz), 7.41 (4H, d, J_{AB} 8.8 Hz), 4.7 (3H, m, $CHCH_2$-Bnpe); δ_c ($CDCl_3$, 50.2 MHz, DEPT) 129.1, 124.1 (aromatic CH's), 68.0 (CH_2), 50.2 (CH); $P^{31}\delta(CDCl_3)$ 178.65; m/z (FAB) H.R.M.S. Found: 387.97829; $C_{14}H_{11}N_2O_5PCl_2$ requires 387.9783 (.˙.=<1 ppm).

Diisopropylamine phosphorus dichloridite (*117*)

To PCl_3 (5 ml, 57.3 mmol) in dry ether (15 ml) at -10°C over argon was added diisopropylamine (16 ml, 1.15 mmol) in diethyl ether (15 ml) over forty-five minutes and left to stir for thirty minutes at room temperature. The copious amount of precipitate was removed by filtration (under 'argon umbrella') to produce a yellow liquid which was re-filtered to produce a clear green liquid. Kugelrohr distillation of this liquid gave a liquid which crystallised on cooling.

(Found: C, 35.5; H, 6.95; N, 7.39; $C_6H_{14}NPCl_2$ requires: C, 35.8; H, 7.0; N, 7.0); δ_H ($CDCl_3$, 200 MHz) 3.9 (2H, m_{AB_6}), 1.25 (12H, d, J 6.8 Hz); m/z (FAB) H.R.M.S. Found: 201.02432, $C_6H_{14}BPCl_2$ requires 201.02409 (.˙.<2 ppm). Mp 102-108°C

P^{31} ($CDCl_3$) 168.19 (before and after distillation).

Morpholino phosphorus dichloridite (*118*)

The procedure for (*117*) was repeated using PCl_3 (57 mmol)

in 20 ml dry ether, morpholine (10 ml, 115 mmol) in 15 ml dry ether. Addition was carried out over thirty minutes and the reaction stirred for thirty minutes at room temperature. Distillation on Kugelrohr under reduced pressure (water pump) product distilled over at 150°C. Mp 25-30°C. ^{31}P ($CDCl_3$), 152.1 ppm, δ_H ($CDCl_3$, 200 MHz) 3.64 (4H, $m_{A_4B_4}$), 3.28 (4H, $m_{A_4B_4}$); m/z (FAB) HRMS; Found: 187.97987; $C_4H_9NOPCl_2$ requires 187.97988 (.˙. = <1 ppm).

Table 3

Sample	observed ratios			
	Leu	Ala	Gly	Val
110	0.97	1.08	0.94	1.02
111	0.90	1.04	1.10	0.99
112	0.94	1.07	1.00	0.99

3.11 Appendixes

(A) Graphs for the kinetic investigation into the elimination of 2,2-bis(4-nitrophenyl)ethyl cinnamate (Bnpe cinnamate).

(B) The crystallographic structure of N-[2,2-bis(4-nitrophenyl)ethoxycarbonyl]-L-alanine as determined by X-ray analysis.

(C) Amino acid chromatograms for the investigation into dimer formation racemisation and purity of Bnpeoc and Fmoc amino acids on p-alkoxybenzyl ester resin.

(D) Circular dichroism spectra for Bnpeoc amino acids.

Appendix A

Figure

(1) Calibration graph for 2,2-bis(4-nitrophenyl) ethyl cinnamate.

(2) Calibration graph for 2,2-bis(4-nitrophenyl) ethyl acetate.

(3) Calibration graph for 1,1-bis(4-nitrophenyl) ethene.

Elimination of Bnpe cinnamate

(4) at 291K with 1 equivalent DBN (first order plot);

(5) at 291K with 1 equivalent DBN (second order plot);

(6) at 281K with 1 equivalent DBN (first order plot);

(7) at 281K with 1 equivalent DNB (second order plot);

(8) at 270K with 1 equivalent DBN (first order plot);

(9) 1t 270K with 1 equivalent DBN (second order plot);

(10) at 256K with 1 equivalent DBN (first order plot);

(11) at 256K with 1 equivalent DBN (second order plot);

(12) at 268K with 2 equivalents DBN;

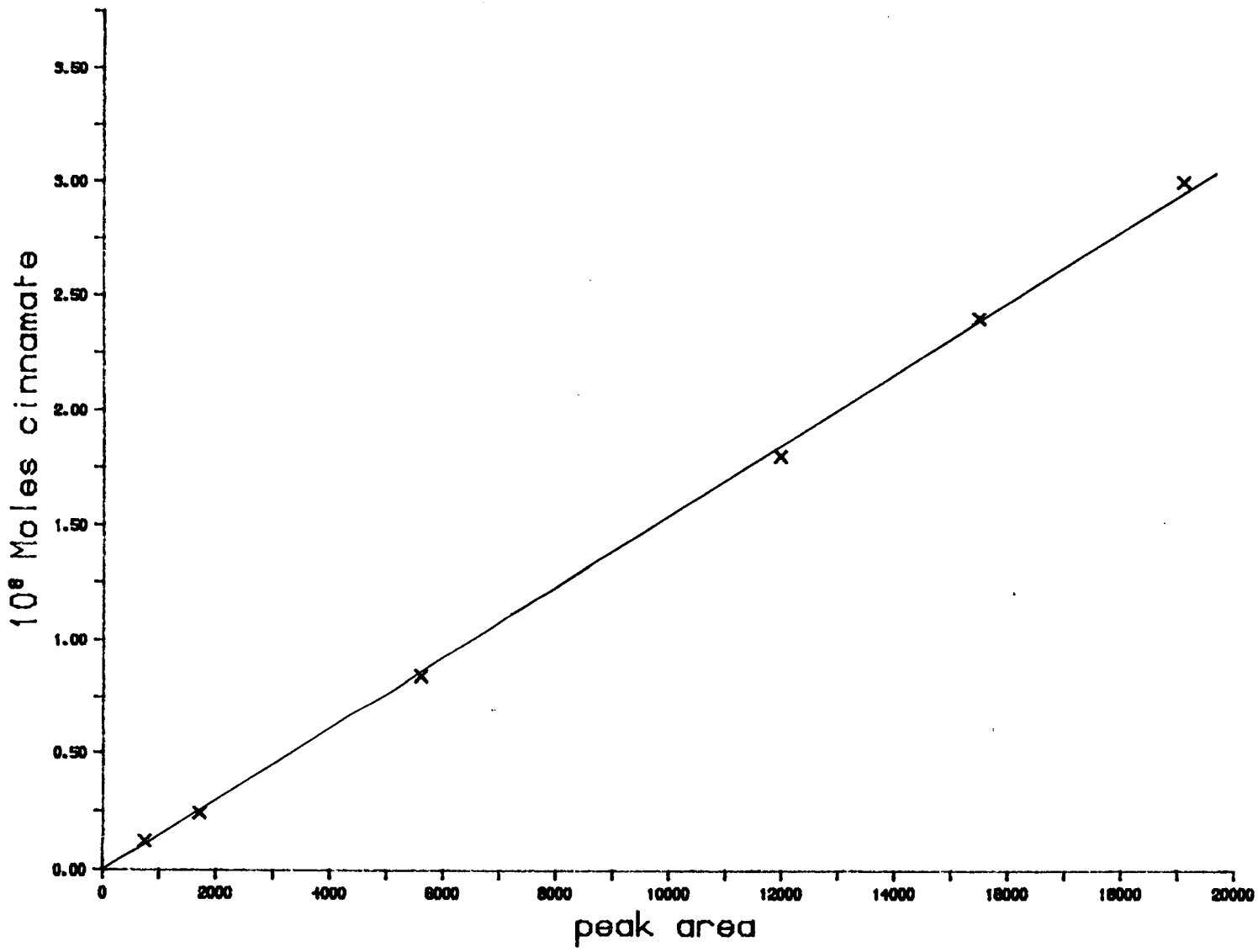
(13) at 268K with 4 equivalents DBN;

(14) Arrhenius plot for 1st order rates;

(15) Arrhenius plot for 2nd order rates.

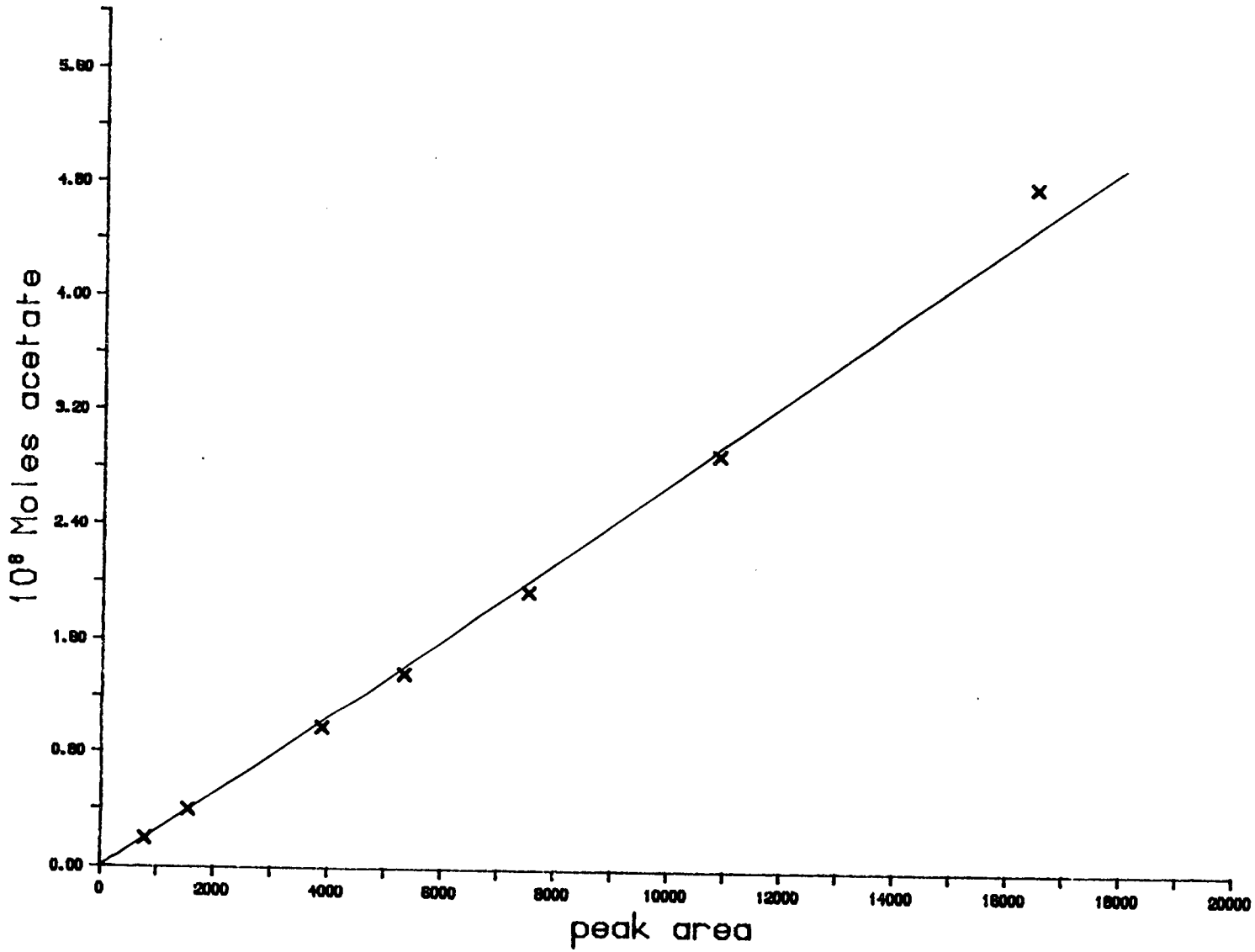
Calibration Graph For

(1) 2,2-Bis(4-nitrophenyl)ethyl cinnamate

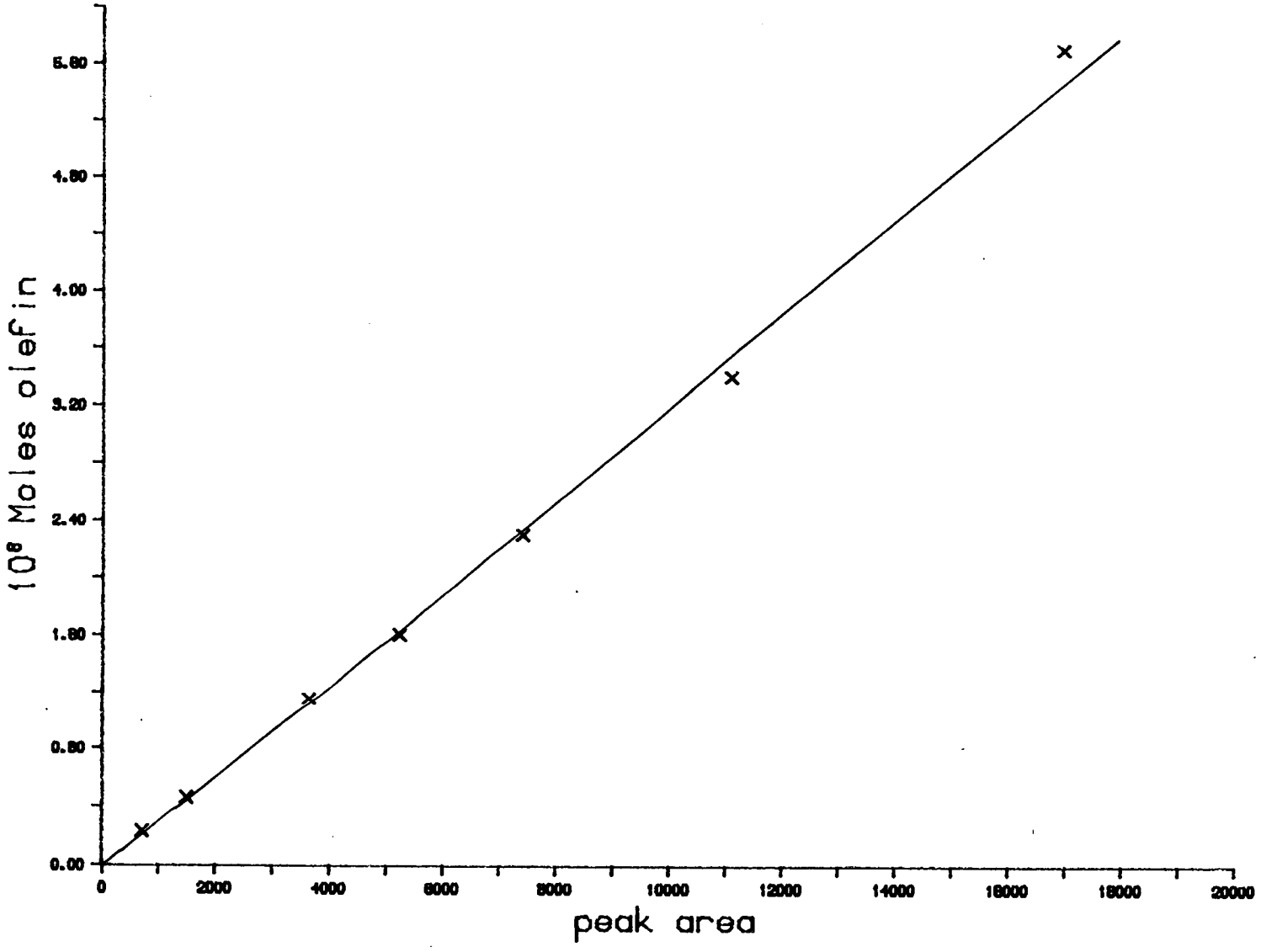


Calibration Graph for

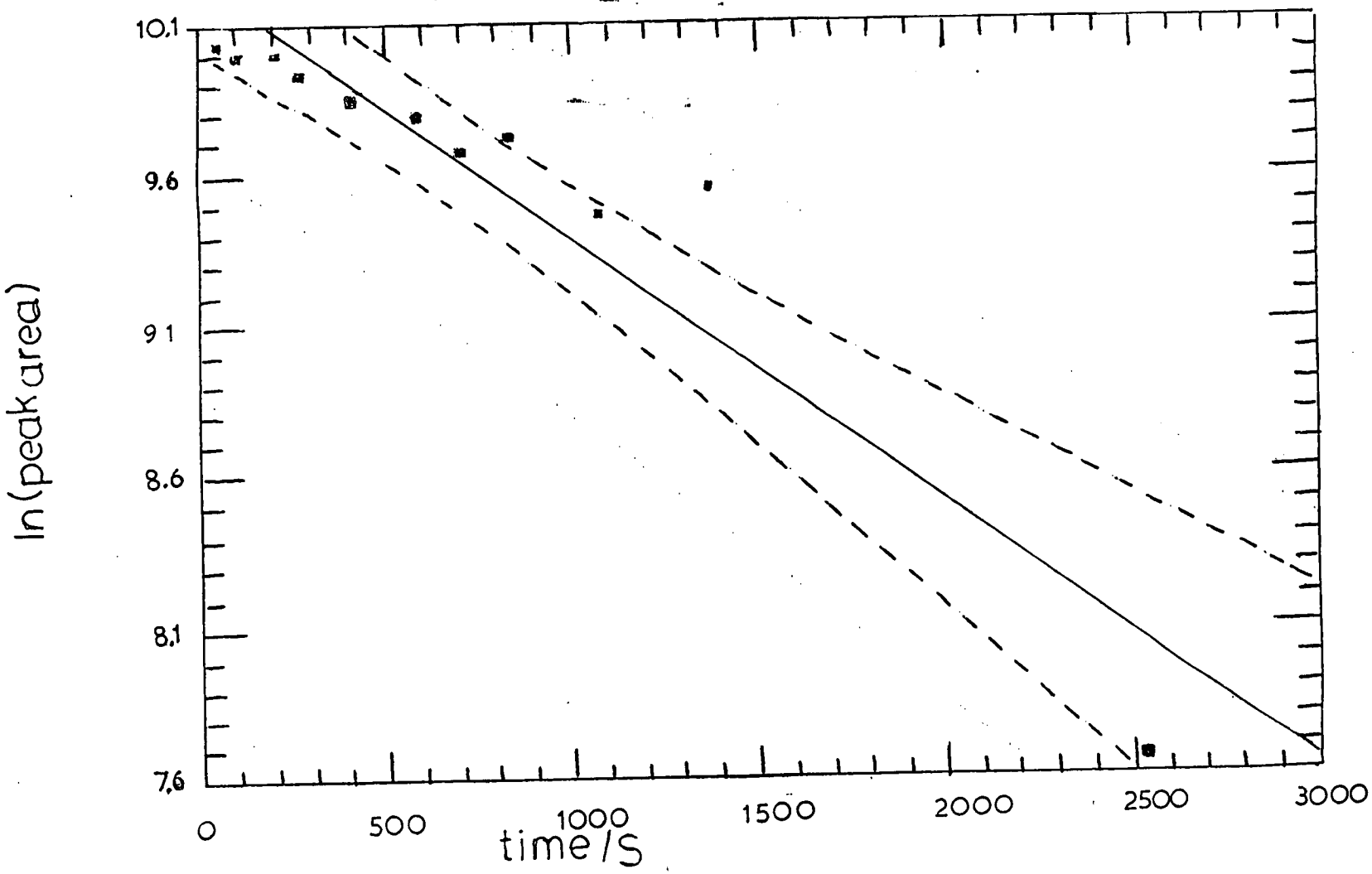
(2) 2,2-Bis(4-nitrophenyl)ethyl acetate



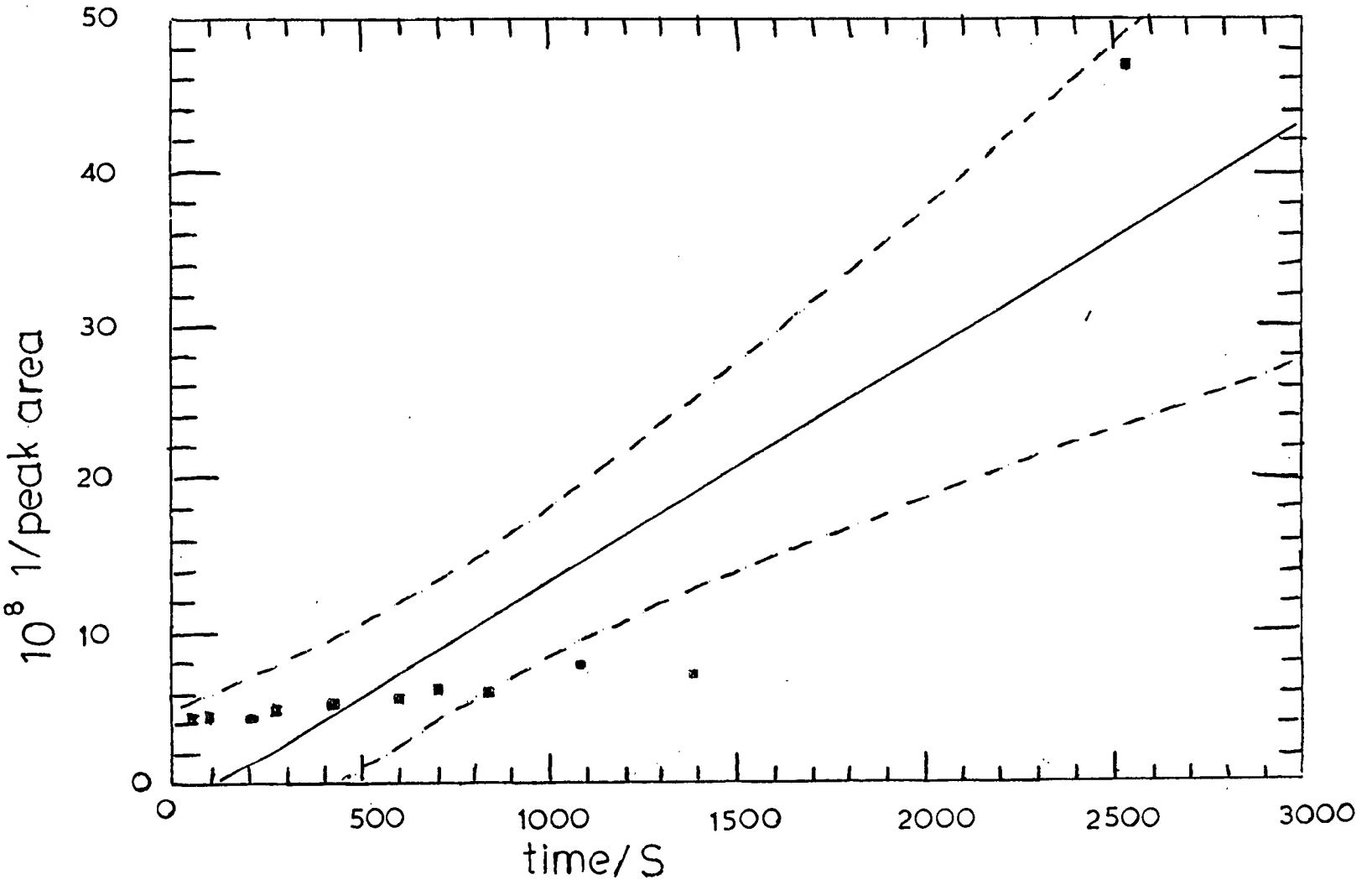
(3) Calibration Graph for 1,1-Bis(4-nitrophenyl)ethene



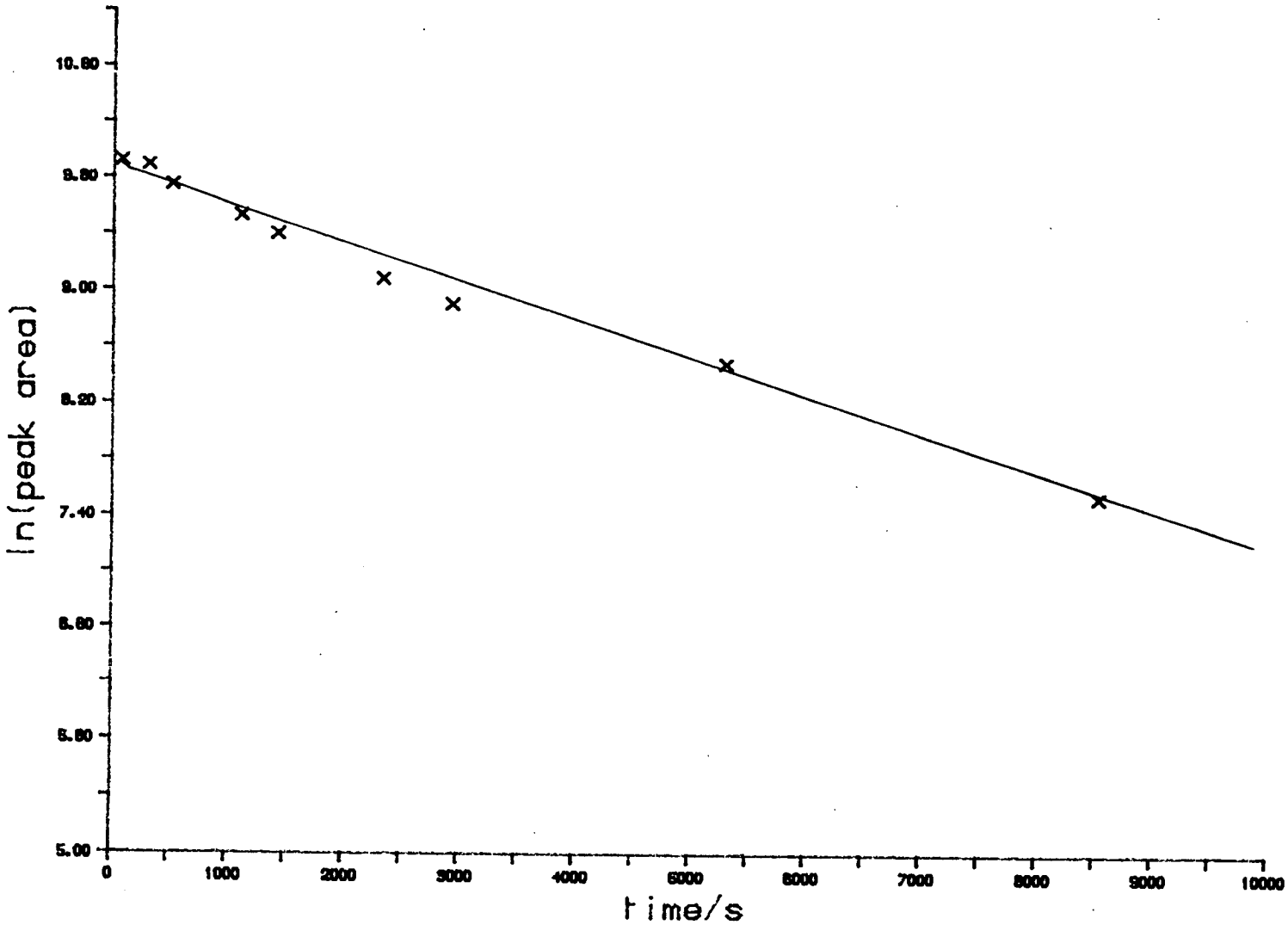
(4) Elimination of Bnpe cinnamate at 291 K with 1 DBN (First order plot)



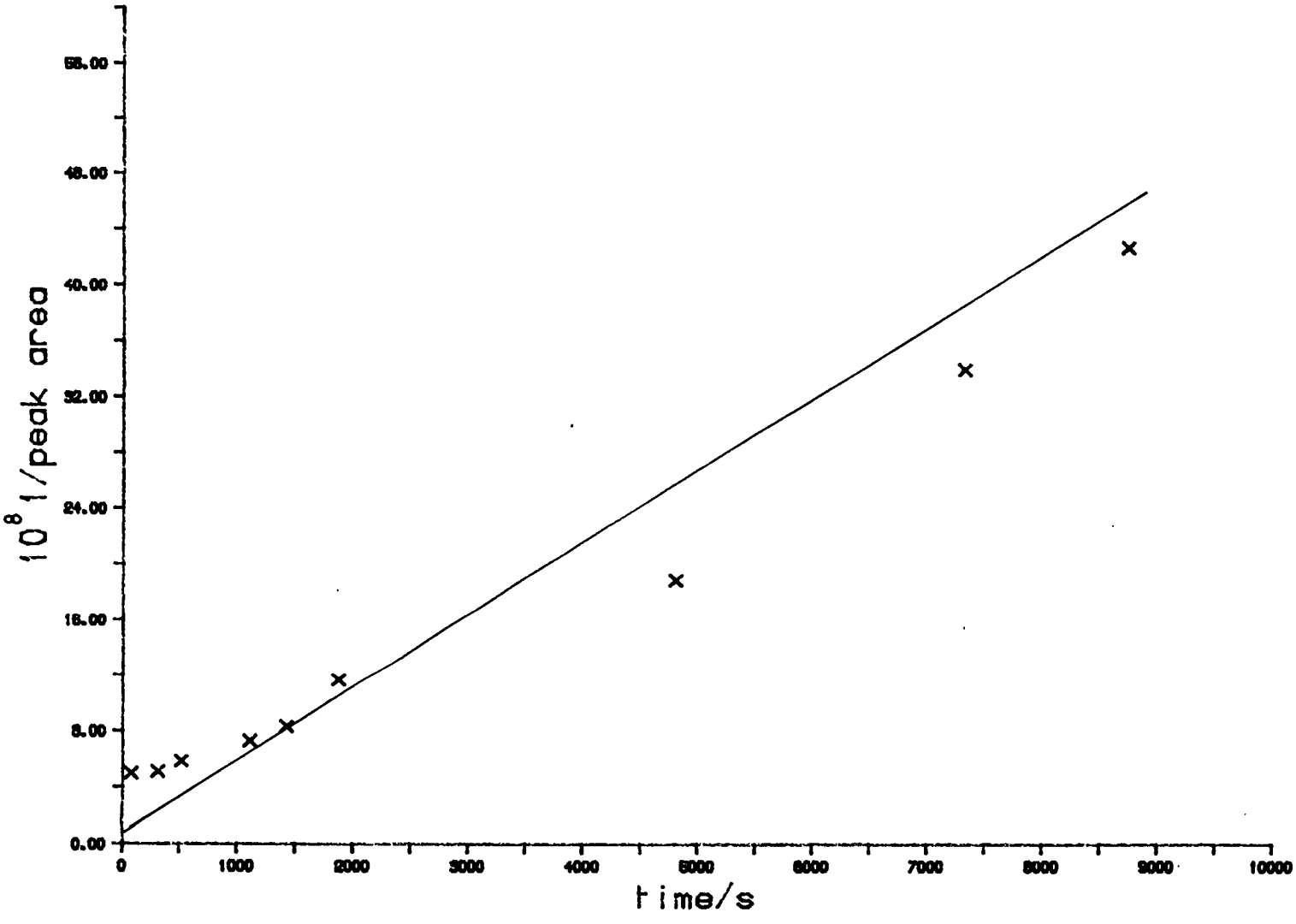
(5) Elimination of Bnpe cinnamate at 291K with 1 DBN (Second order plot)



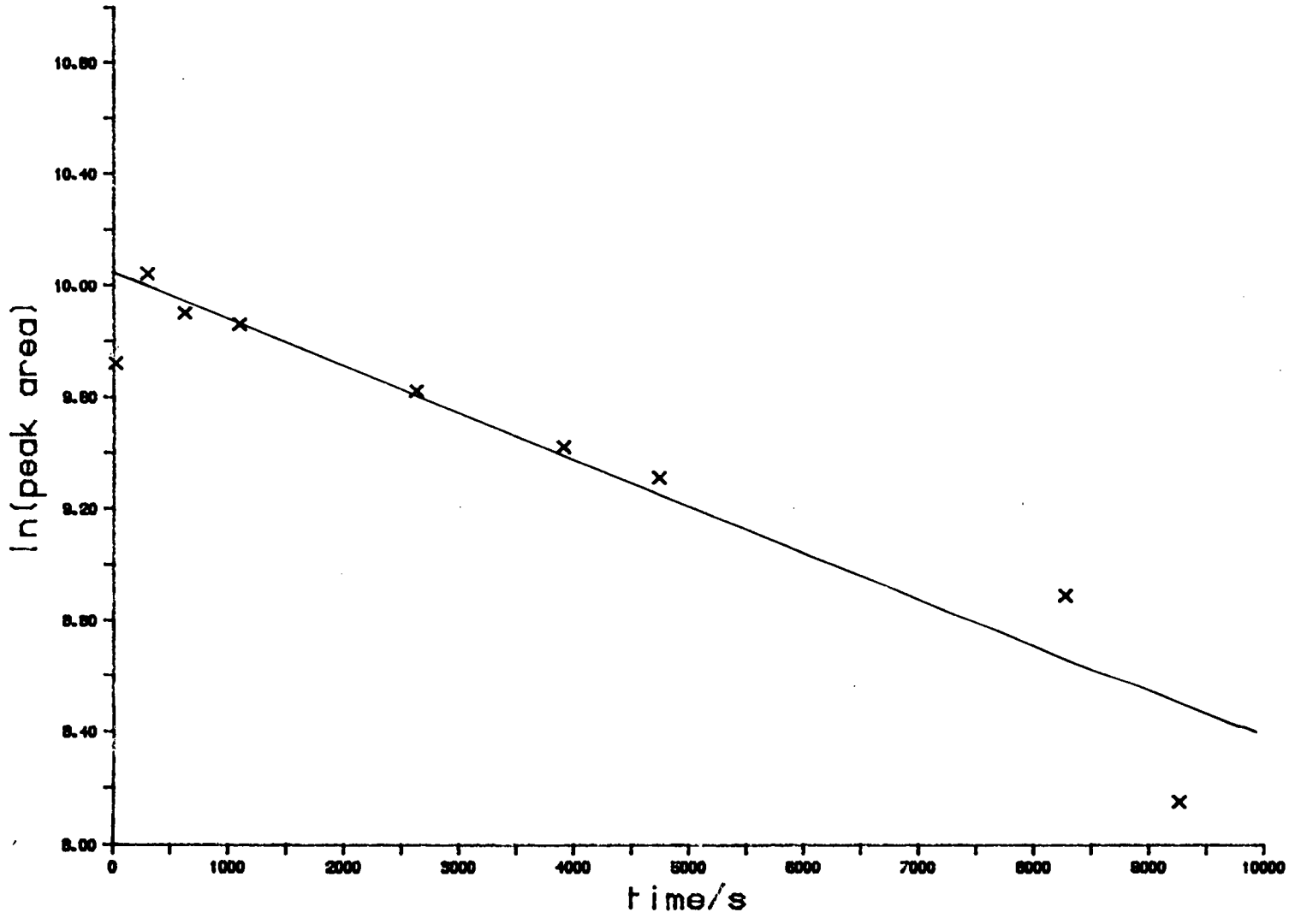
(6) Elimination of Bnpecinnamate at 281K with 1 DBN (First order plot)



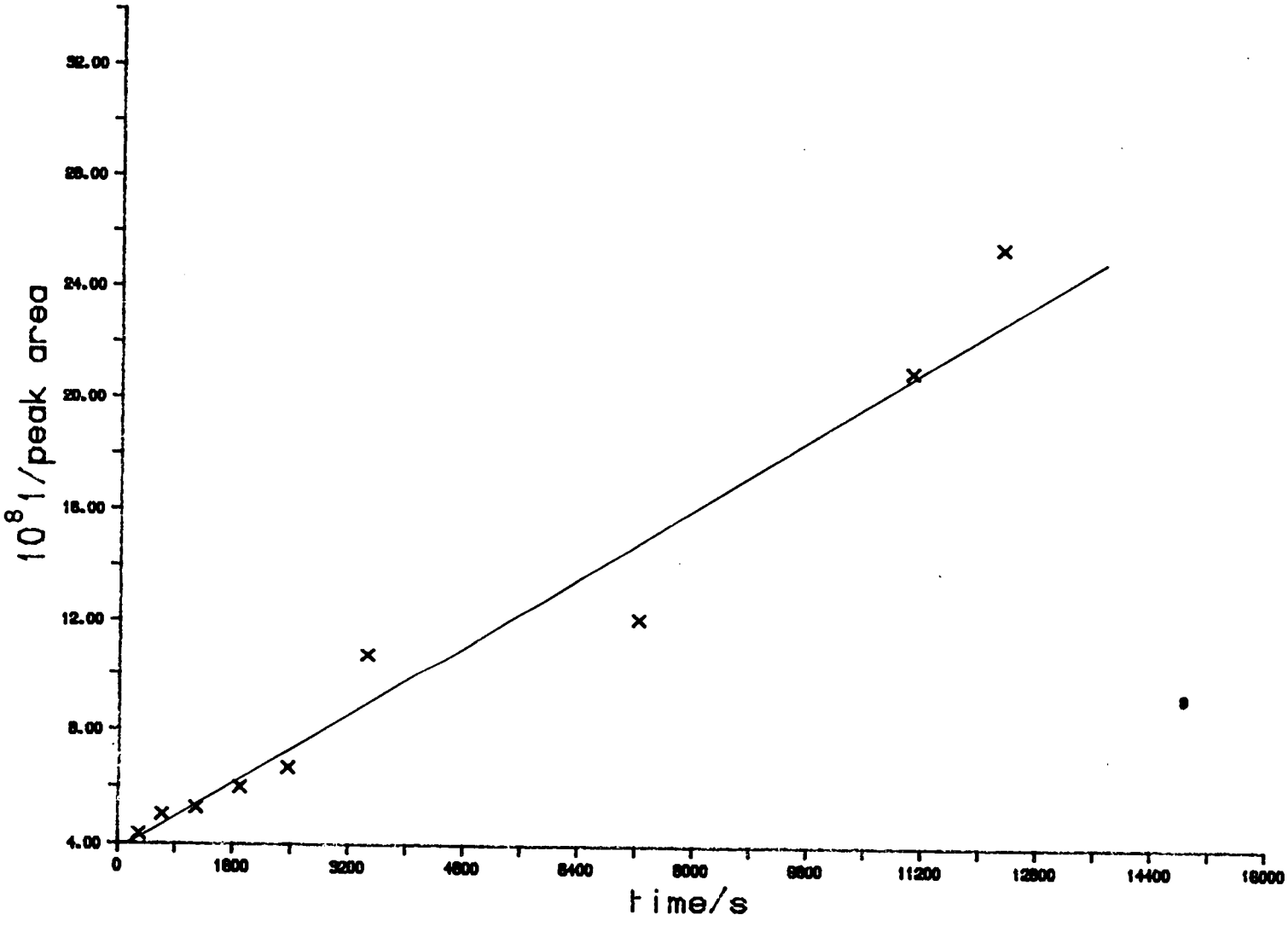
(7) Elimination of Bnpecinnamate at 281K with 1 DBN (Second order plot)



(8) Elimination of Bnpecinnamate at 270K with 1 DBN (First order plot)

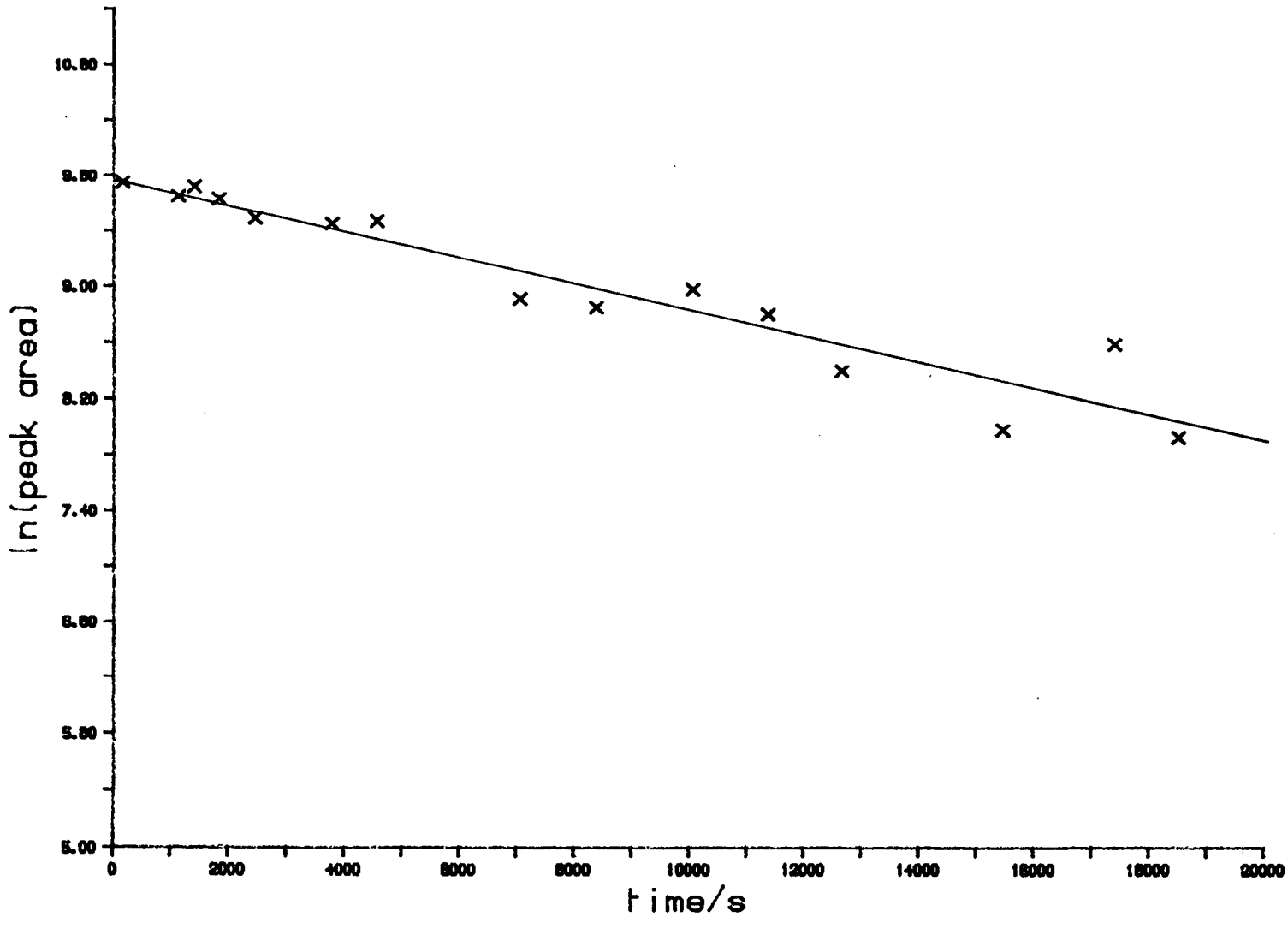


(9) Elimination of Bnpecinnamate at 270K with 1 DBN (Second order plot)

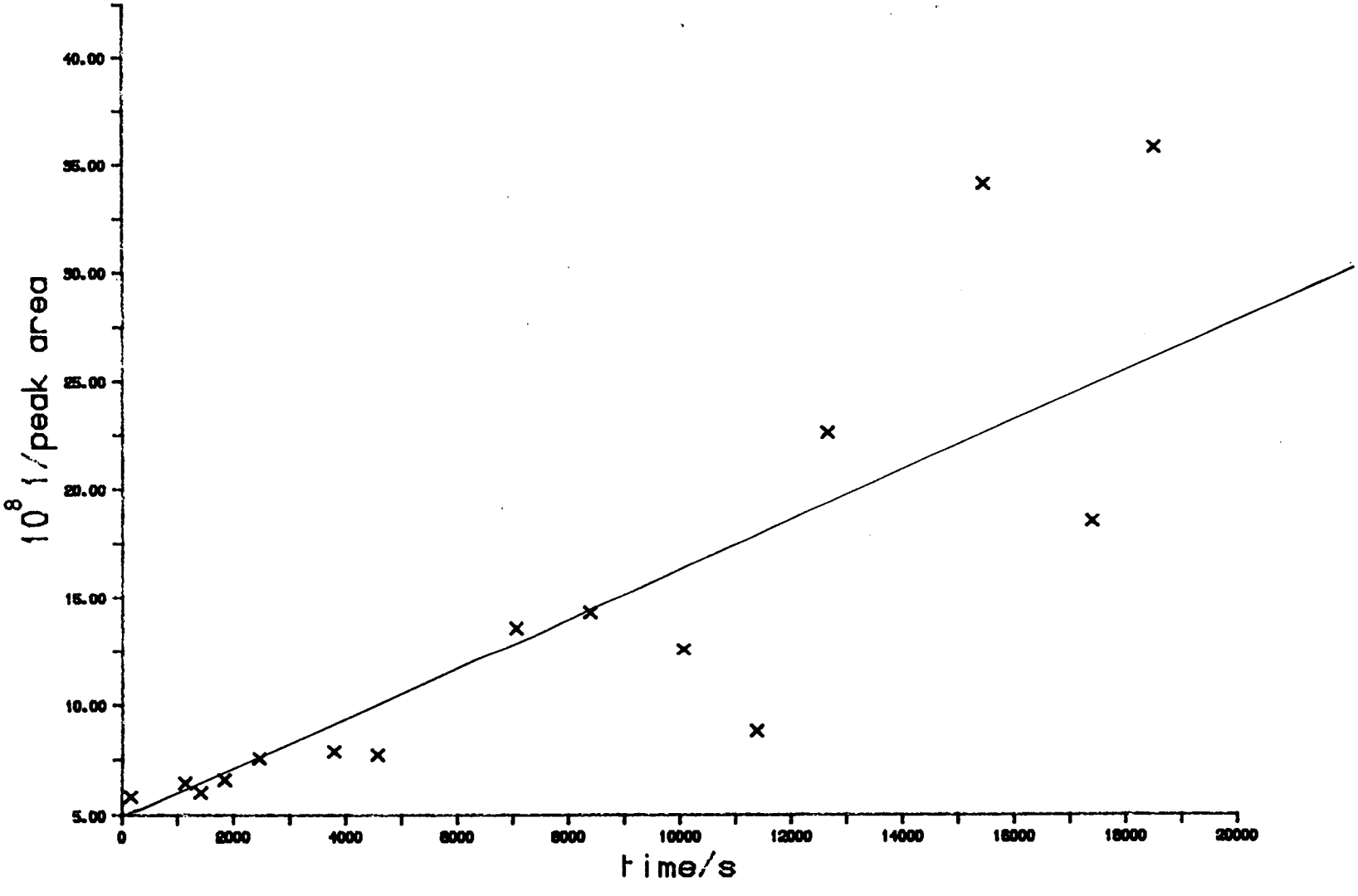


(10)

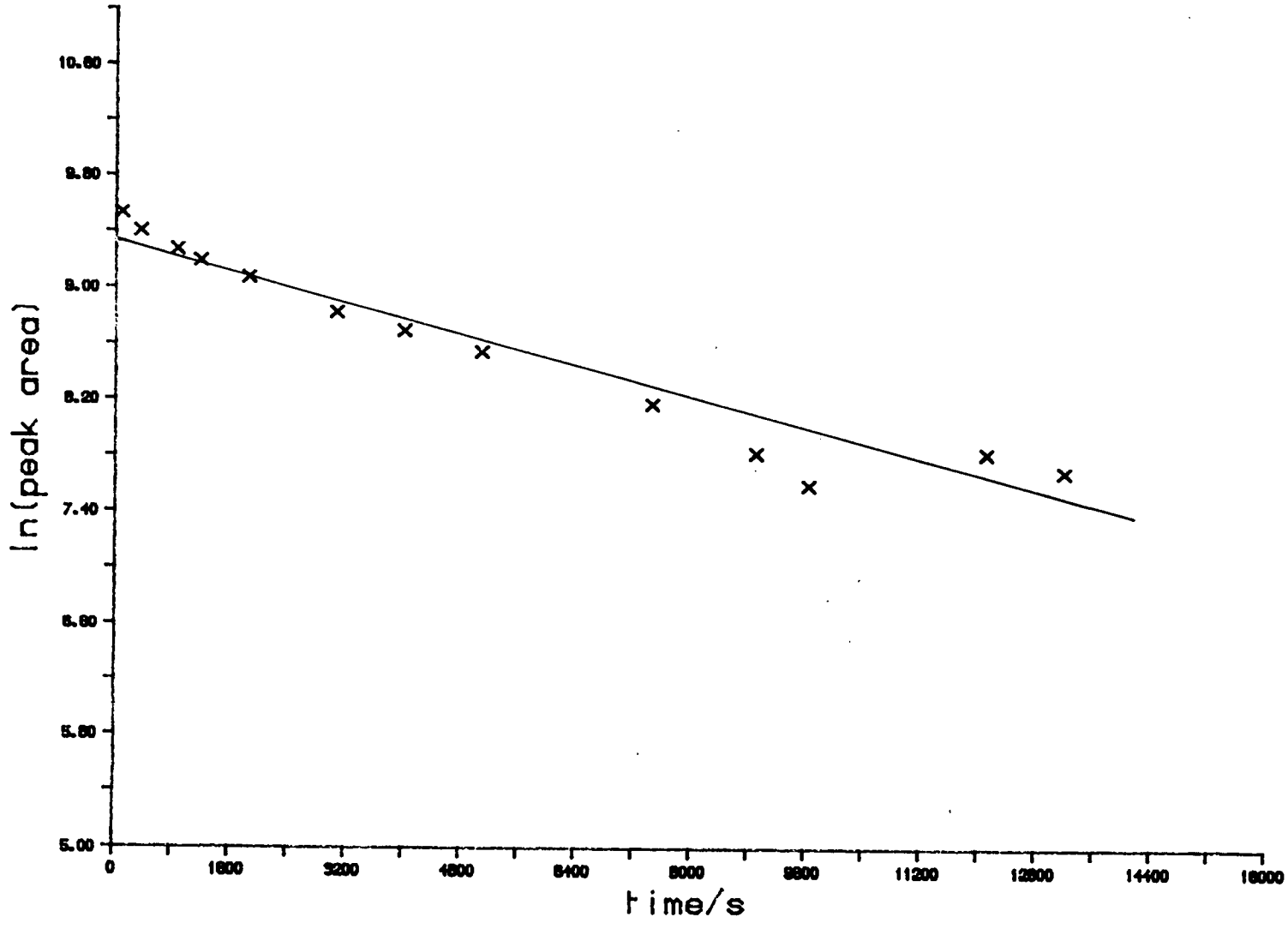
Elimination of Bnpecinnamate at 256K with 1 DBN (First order plot)



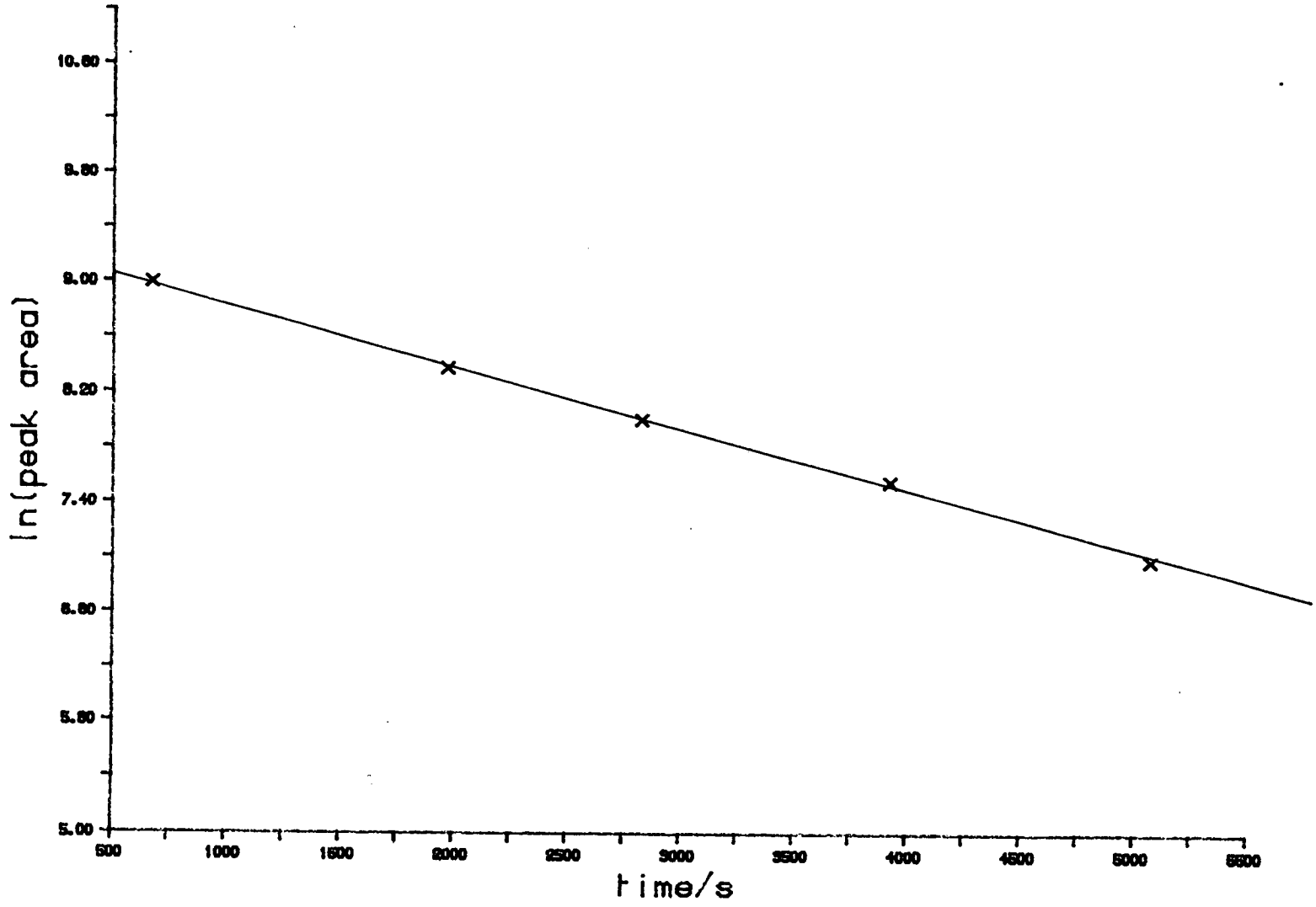
(11) Elimination of Bnpecinnamate at 256K with 1 DBN (Second order plot)



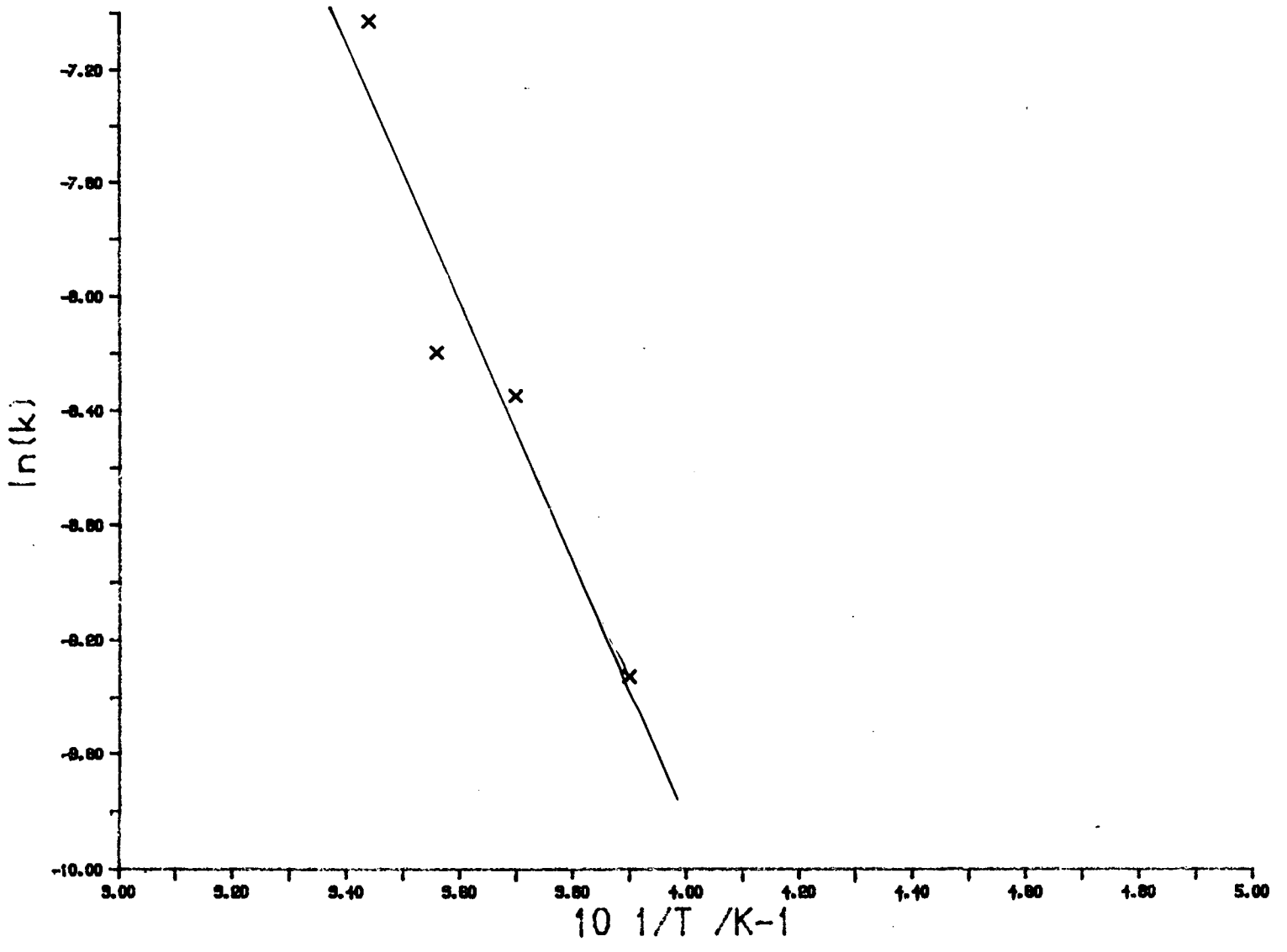
(12) Elimination of Bnpecinnamate at 268K with 2 DBN (First order plot)



(13) Elimination of Bnpecinnamate at 268K with 4 DBN (First order plot)

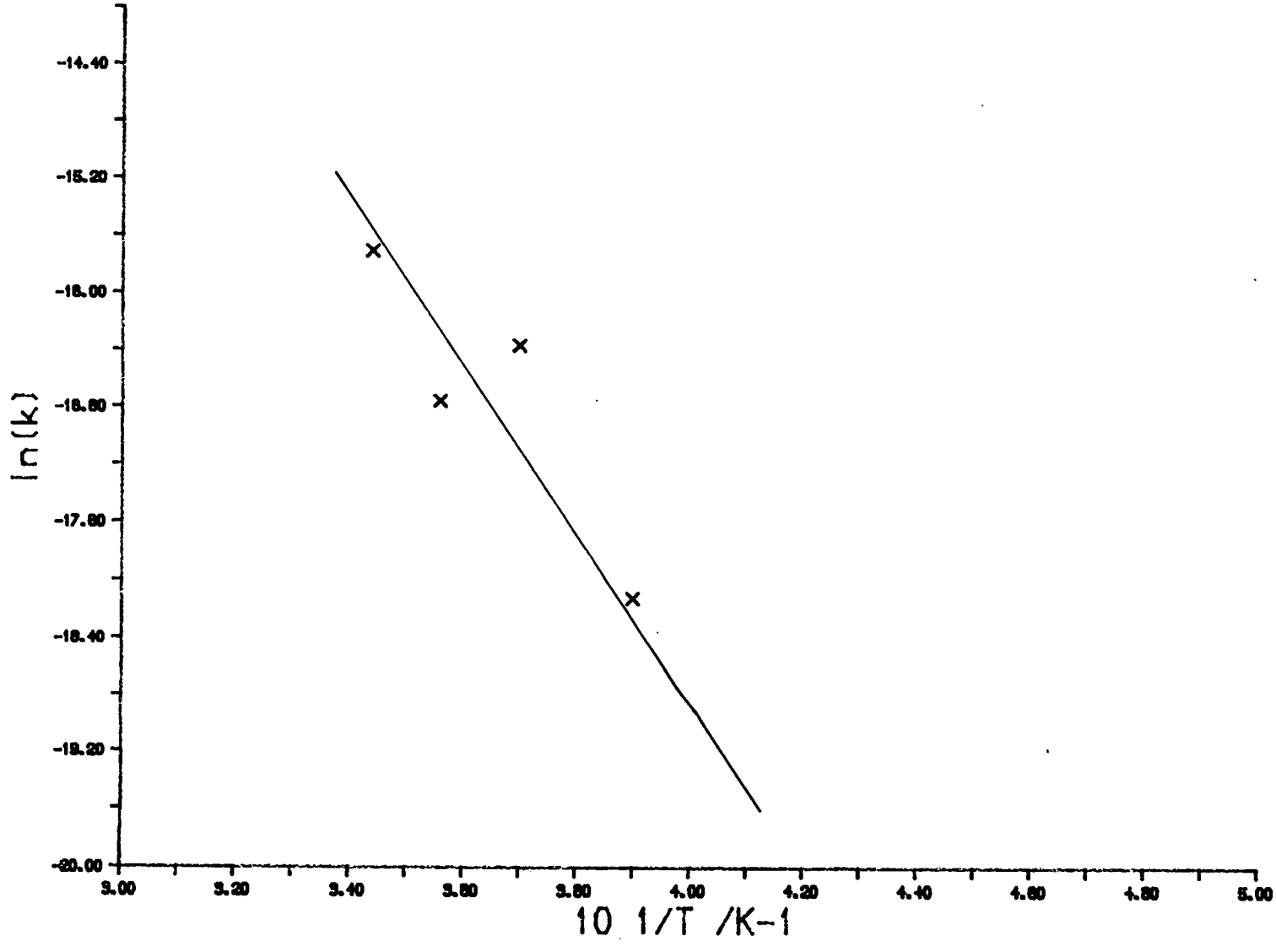


(14) Arrhenius plot for 1st.order rates :
ln(k) vs. 1/T



Arrhenius plot for 2nd.order rates :

(15) ln(k) vs. 1/T



Appendix B

Crystallographic structure of N-[2,2-bis(4-nitrophenyl)ethoxy carbonyl]-L-alanine as determined by X-ray analysis.

(Recorded by Drs. R.O. Gould and A.J. Blake at the University of Edinburgh)

Fig.1: Bnpeoc AlaOH

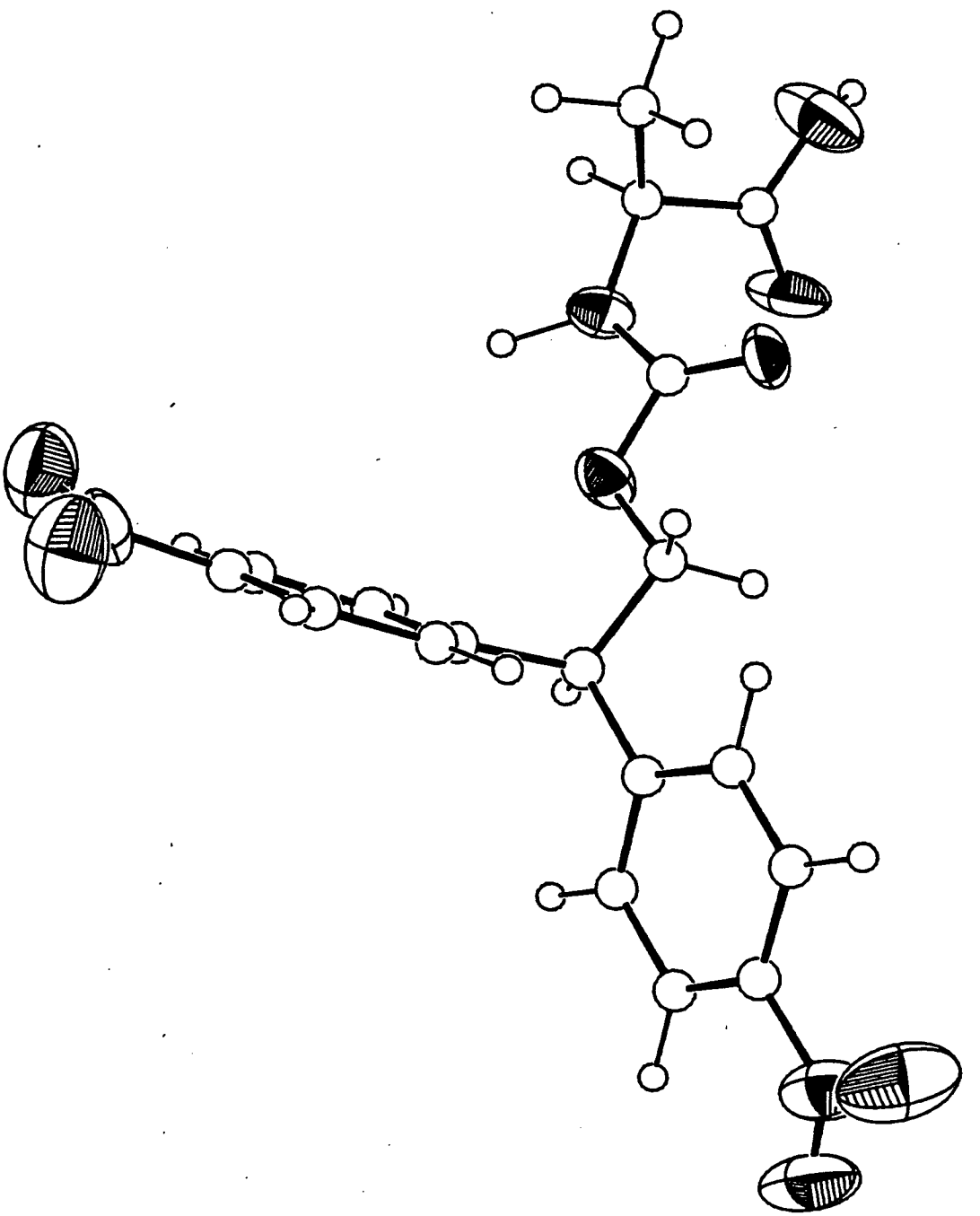
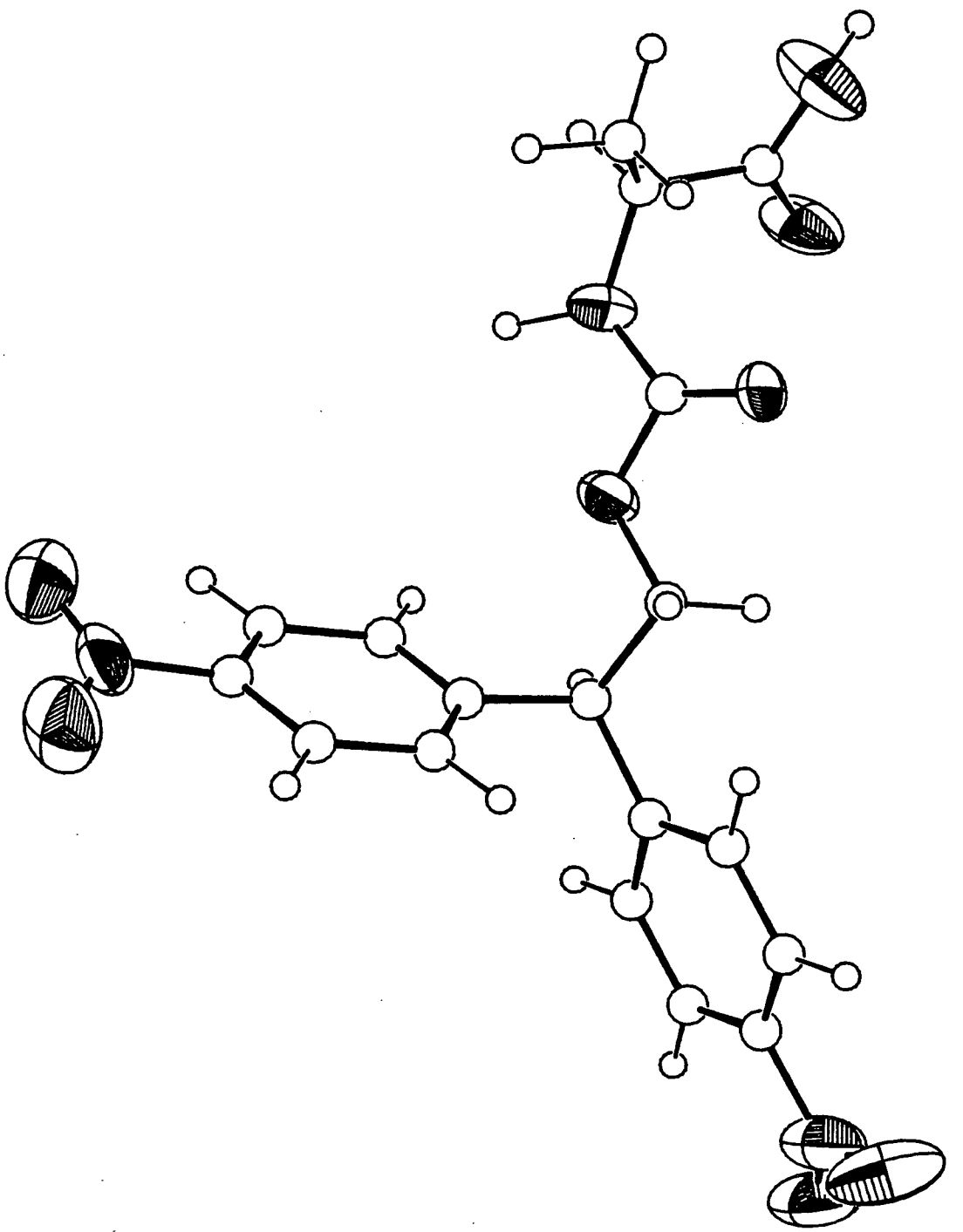


Fig.2: Bnpeoc AlaOH



Appendix C

Figure

(1) Ala (DL) Val (L) standard.

(2) Ala (L) Val (L) standard.

(3) Ala Val dipeptide from resin (98) esterified using Bnpeoc Val-Cl, 5% DMAP method.

(4) Ala Val dipeptide from resin (104) esterifified using Bnpeoc Val-Cl, pyridine method.

(5) Ala Val dipeptide from resin esterified with Fmoc Val-Cl, pyridine.

(6) Gly standard.

(7) GlyGly standard.

(8) Gly and GlyGly content of resin (97) esterified using Bnpeoc Gly-Cl, 5% DMAP method.

(9) Gly and GlyGly content of resin esterified using Fmoc Gly-Cl, 5% DMAP.

(10) Gly and GlyGly content of resin esterified using Bnpeoc Gly-Cl, pyridine

(11) Gly and GlyGly content of resin esterified using Fmoc Gly-Cl, pyridine.

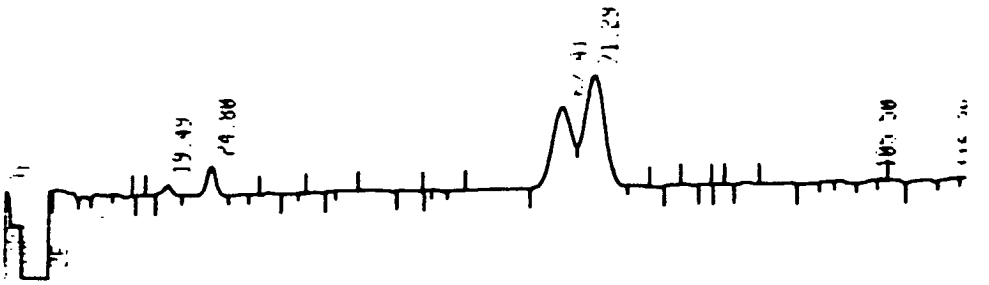
Purity analysis of Merrifield tetrapeptide LeuAlaGlyVal

(12) Synthesised starting with Bnpeoc Val symmetrical anhydride and p-alkoxybenzylalcahol resin.

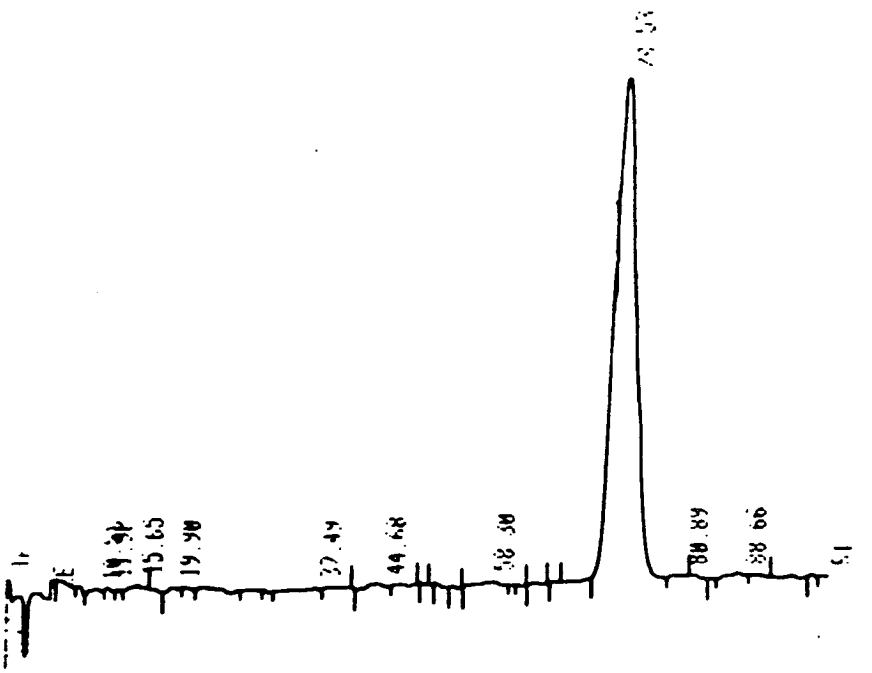
(13) Synthesised starting from Bnpeoc Val-p-alkoxy benzyl ester resin.

(14) Crystallised material from (13).

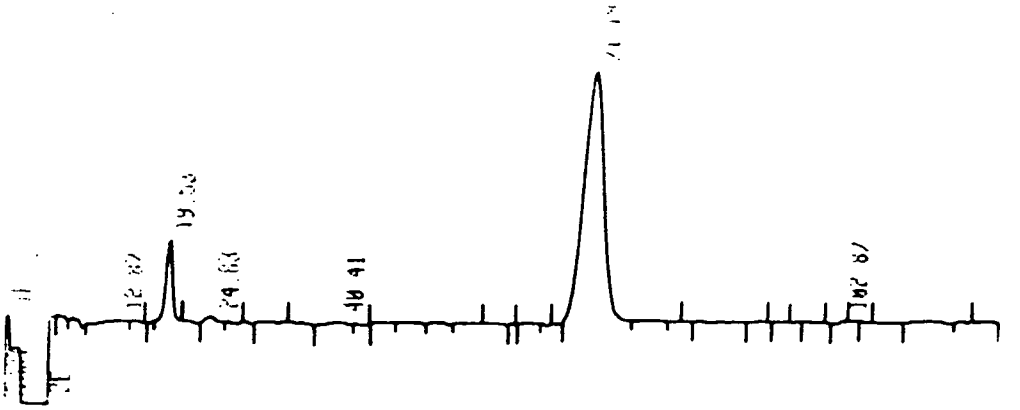
(1) Ala (DL) Val (L) standard



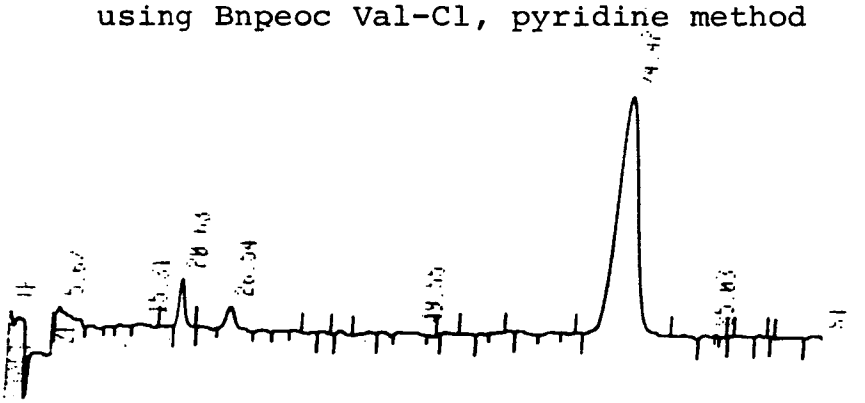
(2) Ala (L) Val (L) standard



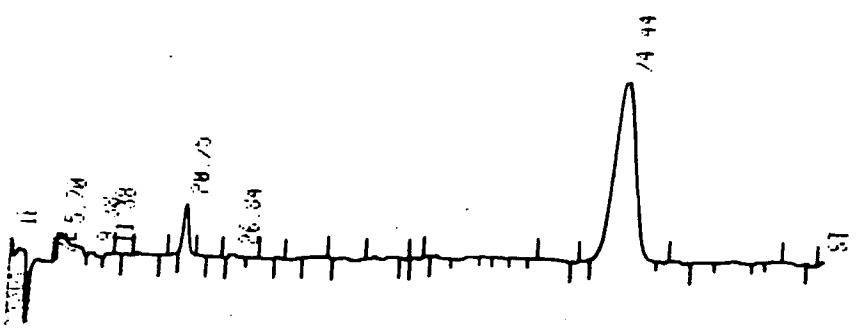
(3) Ala Val dipeptide from resin (98) esterified using Bnpeoc Val-Cl, 5% DMAP method



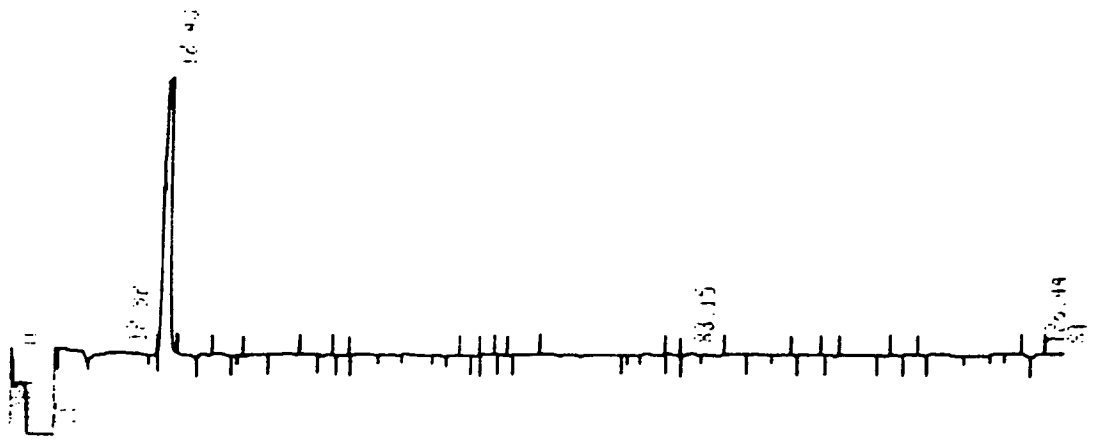
(4) Ala Val dipeptide from resin (104) esterified using Bnpeoc Val-Cl, pyridine method



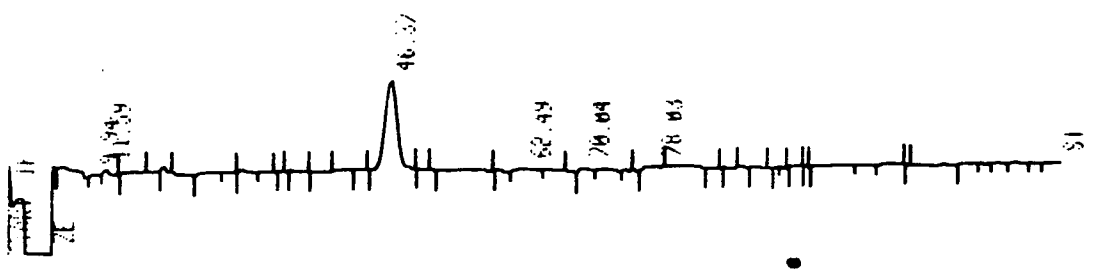
(5) Ala Val dipeptide from resin esterified with Fmoc Val-Cl, pyridine



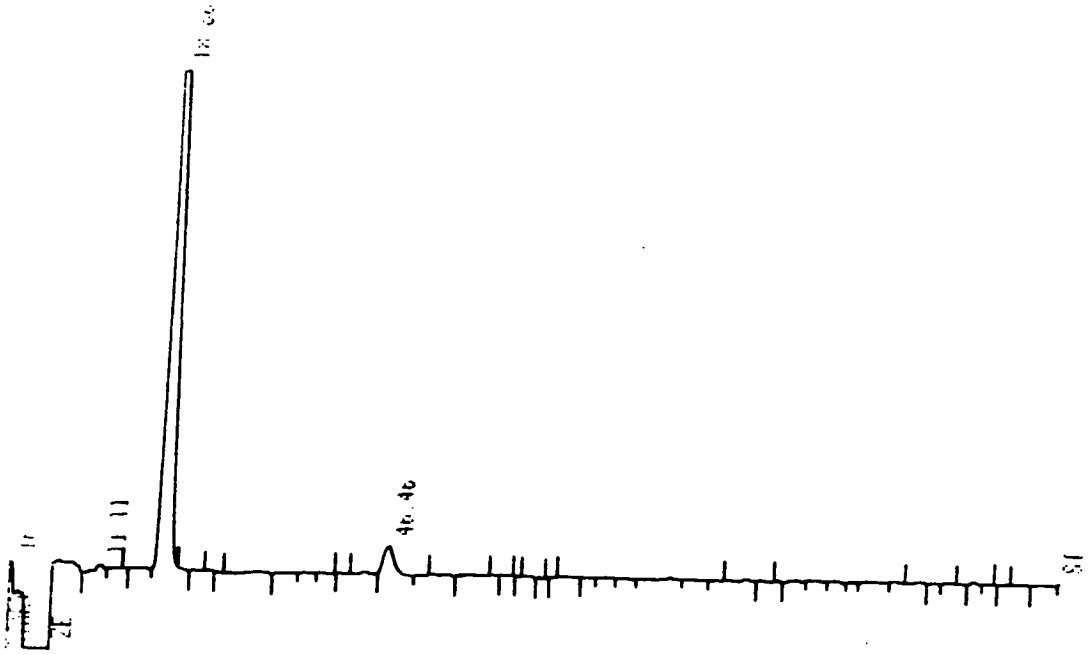
(6) Gly standard



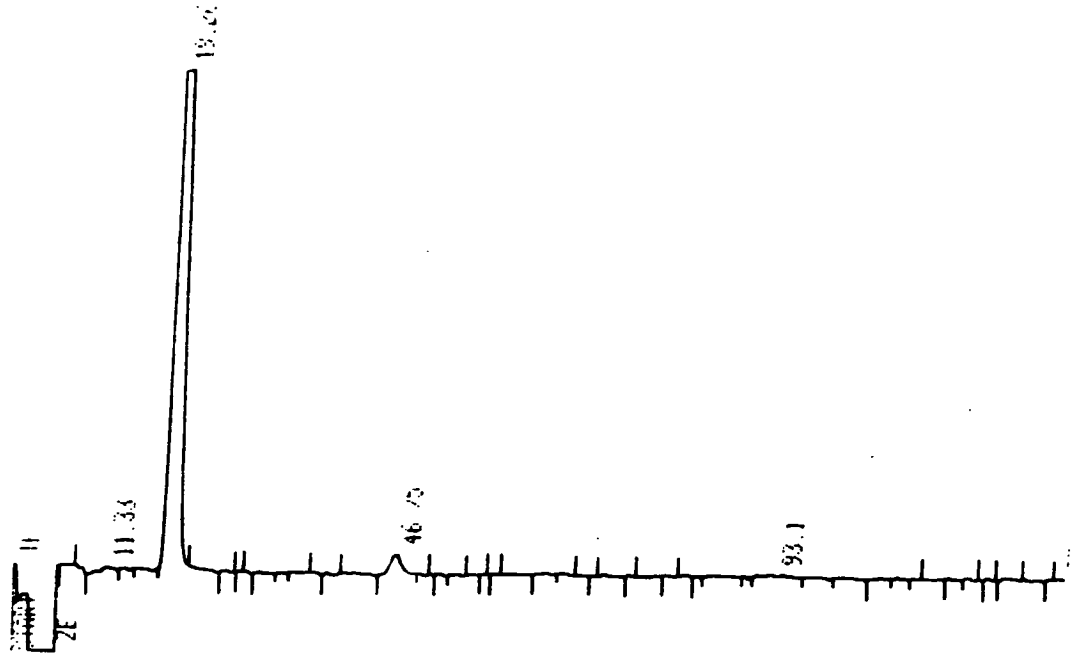
(7) GlyGly standard



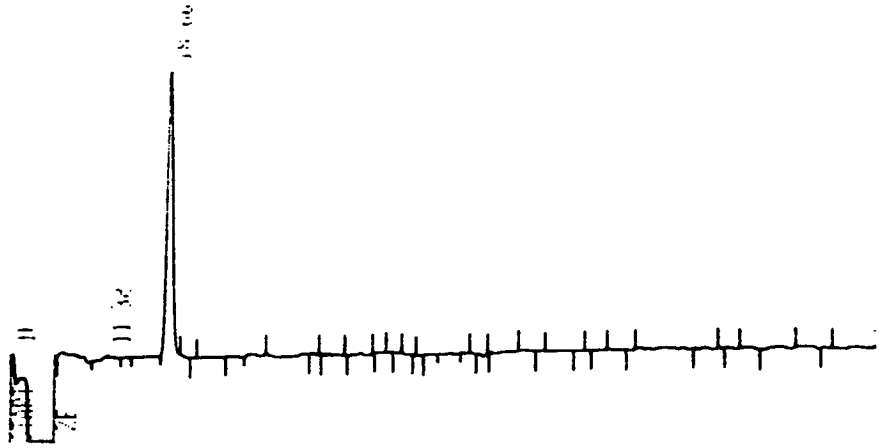
(8) Gly and GlyGly content of resin (97) esterified using Bnpeoc Gly-Cl, 5% DMAP method



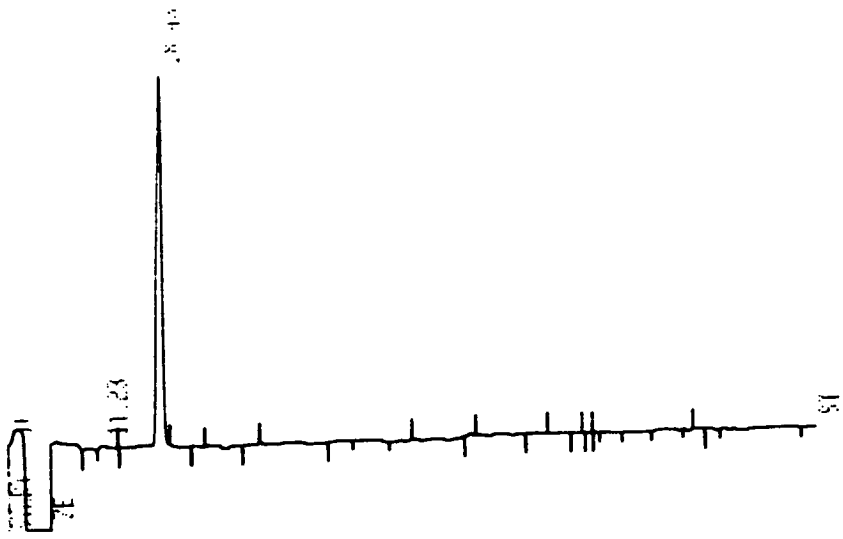
(9) Gly and GlyGly content of resin esterified using Fmoc Gly-Cl, 5% DMAP



(10) Gly and GlyGly content of resin esterified using Bnpeoc Gly-Cl, pyridine



(11) Gly and GlyGly content of resin esterified using Fmoc Gly-Cl, pyridine



Purity analysis of Merrifield tetrapeptide; LeuAlaGlyVal

Synthesised starting with Bnpeoc Val symmetrical anhydride and p-alkoxybenzylalcohol resin

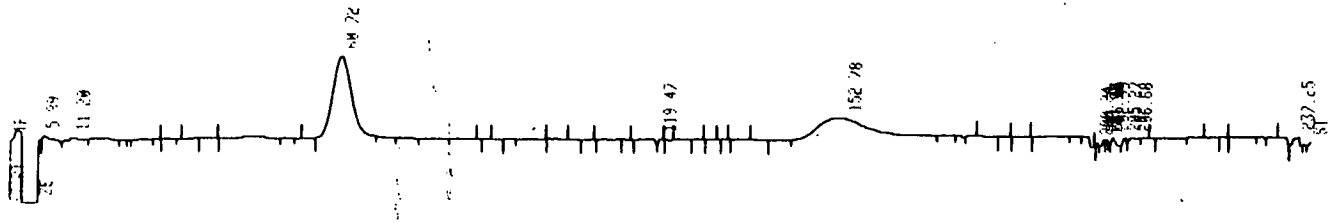


Fig. 12

Synthesised starting from Bnpeoc Val-p-alkoxybenzyl ester resin

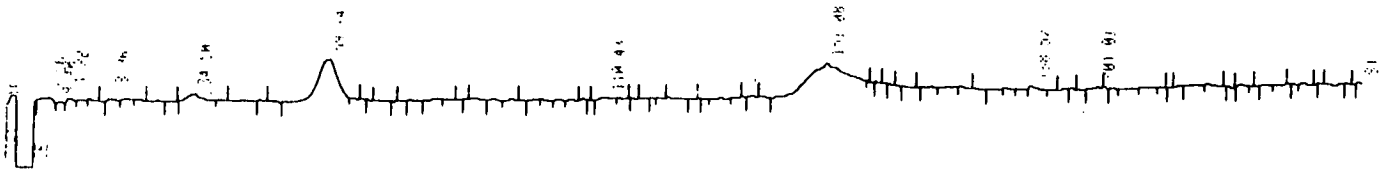


Fig.13

Crystallised material from (13)

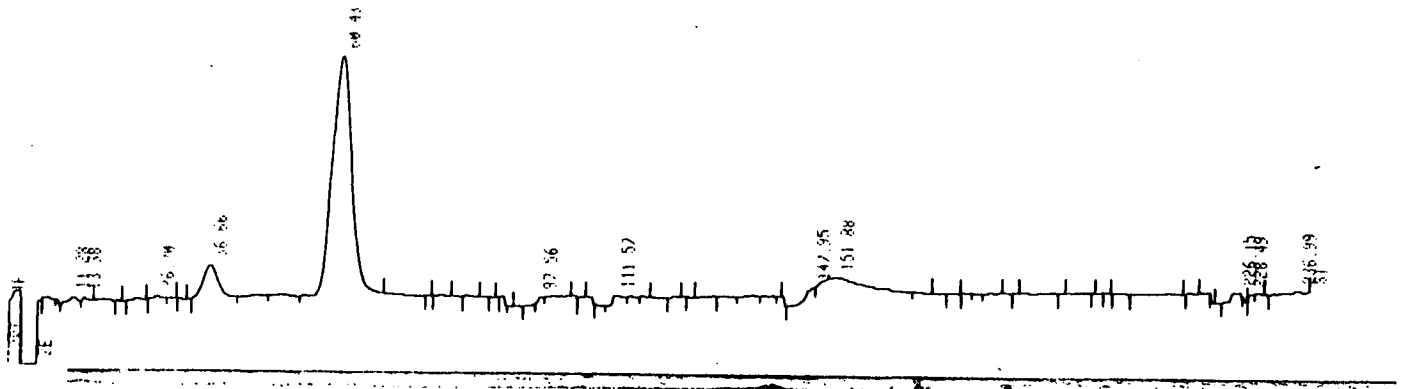
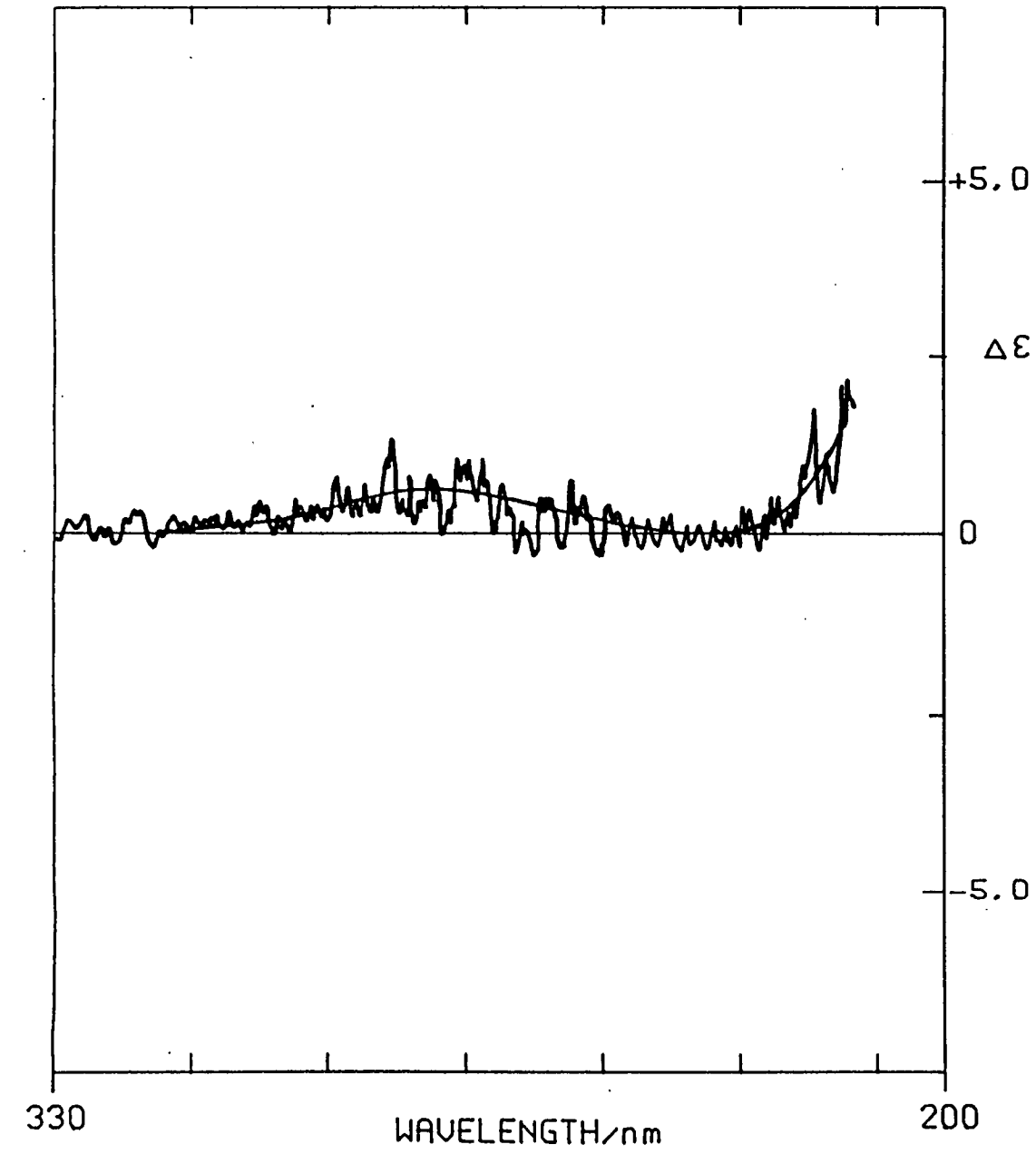


Fig.14

Appendix D

Circular Dichroism Spectra for Bnpeoc amino acids

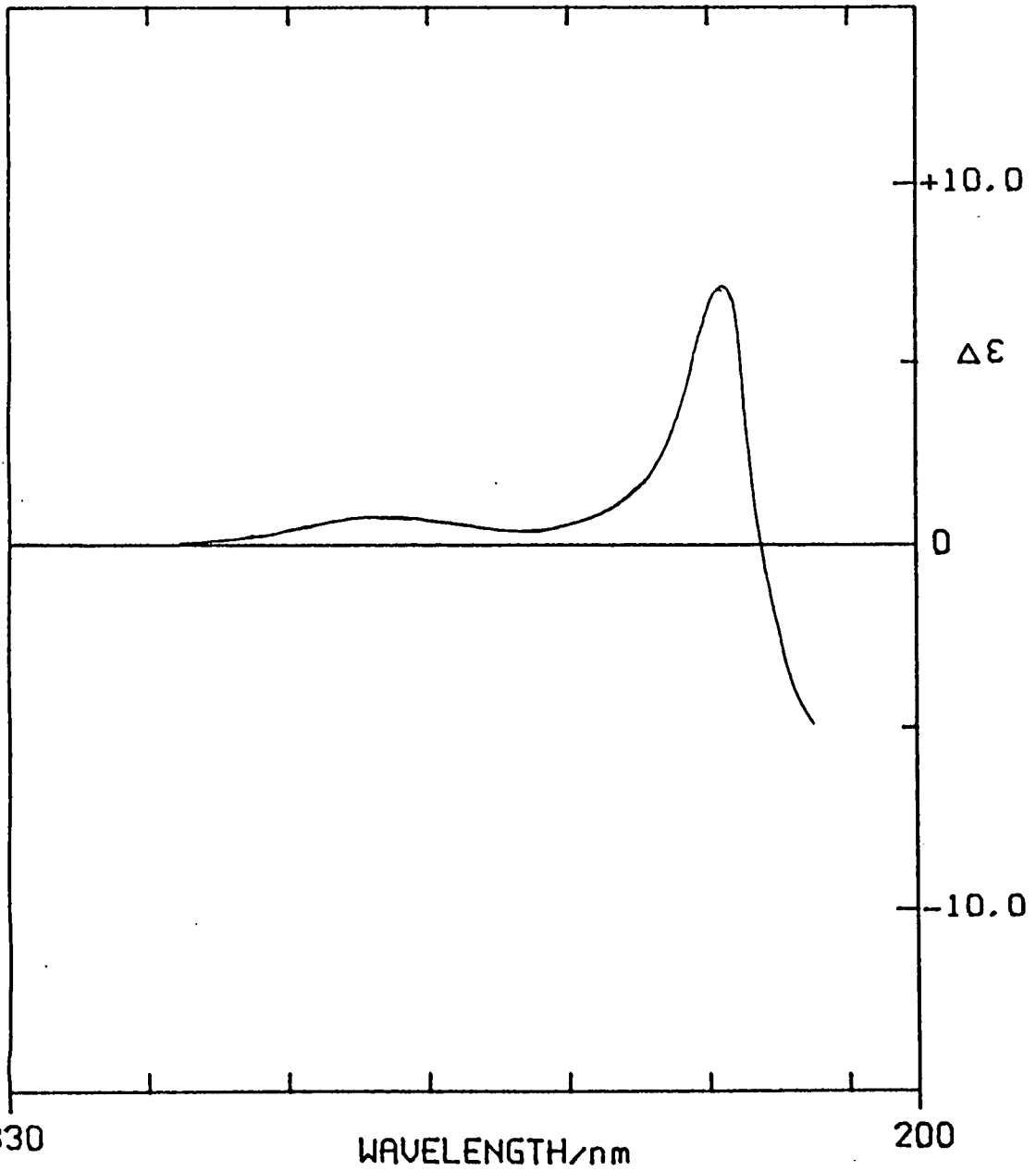
(Recorded by A.F. Drake and P.M. Udvarhelyi at Birbeck College, London)



Samples: EU 1-10, 13, 14

Solvent: CH_3CN

Fig 1

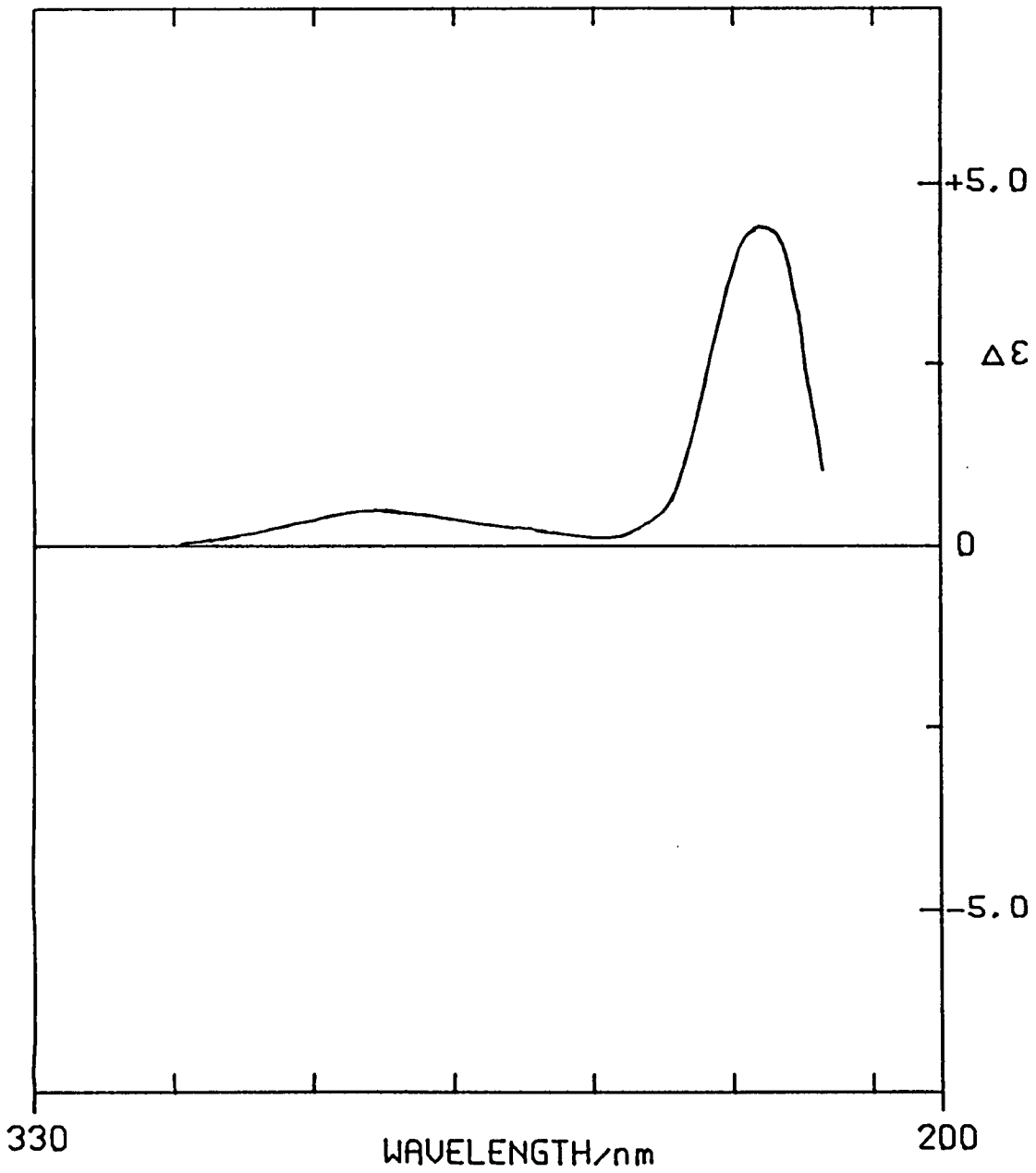


Sample EU11

Bnpeoc TrpOH

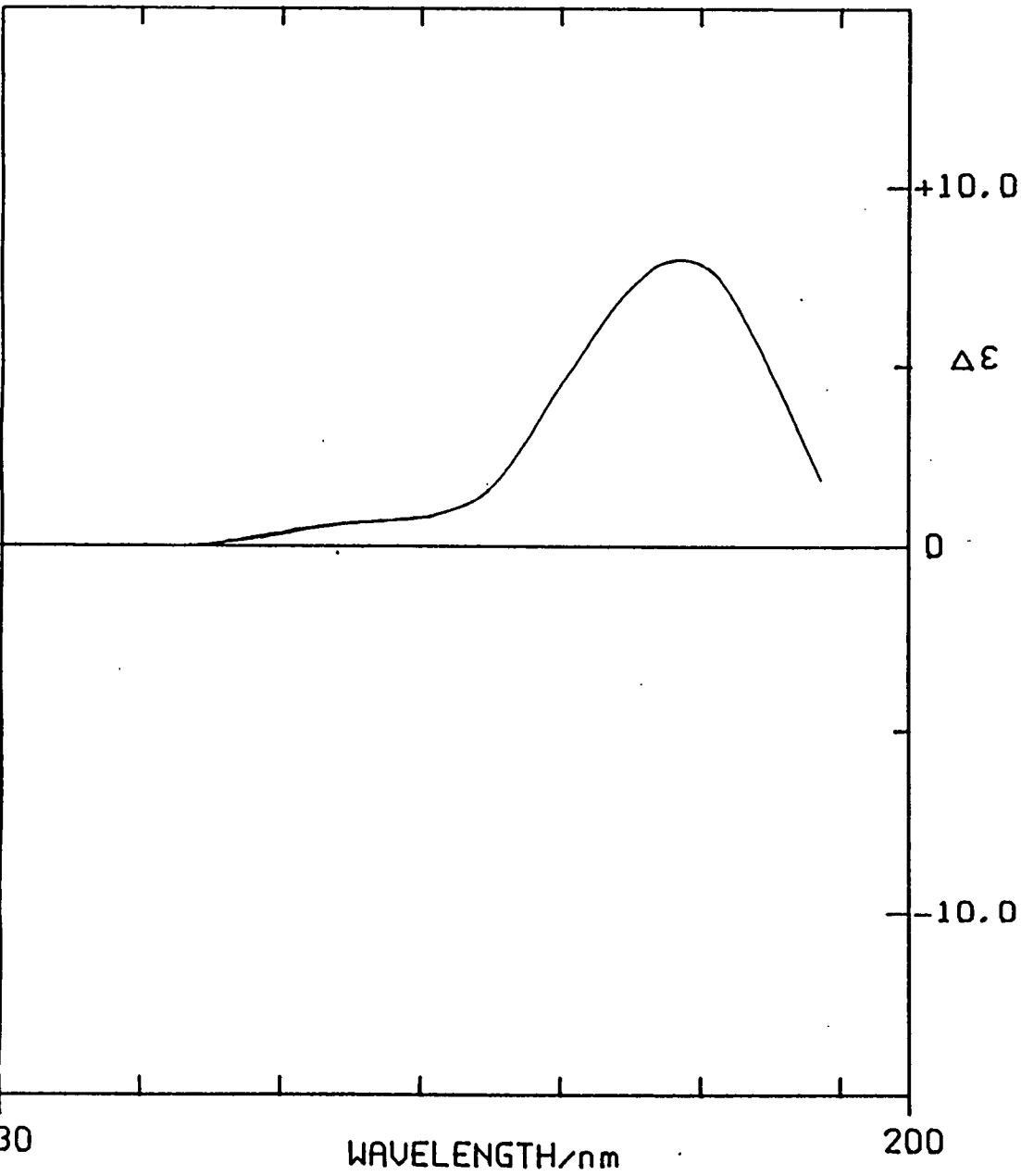
Solvent: CH_3CN

Fig. 2



Sample EU 12

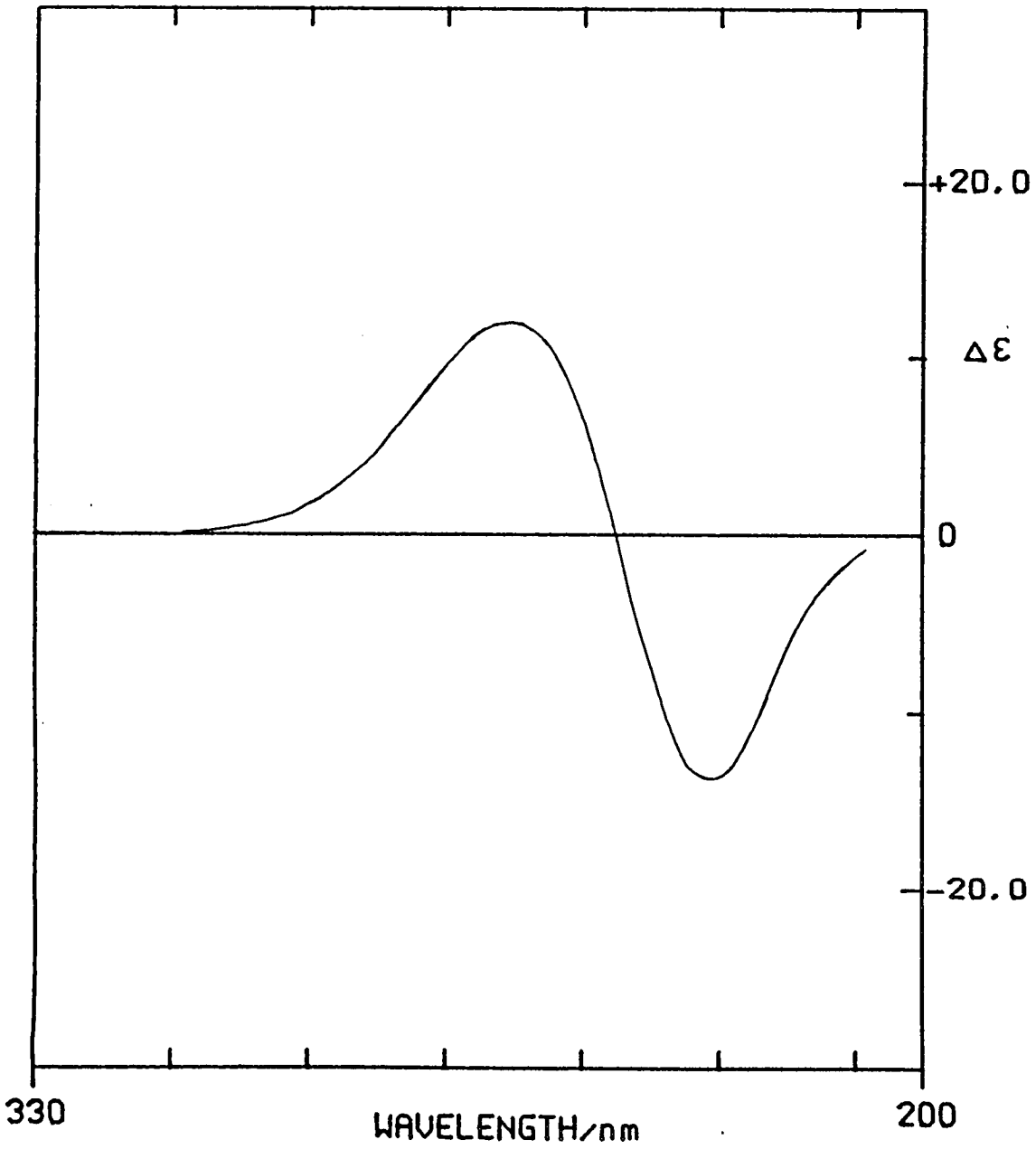
Bnpeoc TyrOH
Solvent: CH_3CN



Sample EU15

Bnpeoc APA OH

Solvent: CH_3CN



Sample EU 16

Bnpeoc ACA OH

Soluent: CH_3CN

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